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Proceedings of the third canadian workshop on harmful marine algae

J.-C. Therriault and M. Levasseur (Editors)

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G5H 3Z4

December 1992

Canadian Technical Report of Fisheries and Aquatic Sciences 1893

Fisheries and Oceans   Pêches et Océans

Canada

93-02601/

Canadian Technical Report of Fisheries and Aquatic Sciences

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Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences
No. 1893

December 1992

PROCEEDINGS OF THE THIRD CANADIAN WORKSHOP ON HARMFUL MARINE ALGAE

Maurice-Lamontagne Institute
Mont-Joli, Québec
12-14 May, 1992

Edited by

Jean-Claude Therriault & Maurice Levasseur

Ministère des Pêches et des Océans
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Cat. No. Fs 97-6/1893E ISSN 0706-6457

Correct citation for this publication:

Therriault, J.-C. and M. Levasseur 1992. Proceedings of the Third Canadian Workshop on Harmful Marine algae. Maurice Lamontagne Institute, Mont-Joli, Québec, May 12-14, 1992. Can. Tech. Rep. Fish. Aqua. Sci. 1893: 154p.

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RÉSUMÉ

Le Troisième Atelier Canadien sur les Algues Marine Nuisibles a été organisé par le Ministère des Pêches et des Océans, région du Québec, à l'Institut Maurice-Lamontagne, Mont-Joli, du 12 au 14 mai 1992. Quelques 80 participants canadiens et étrangers ont assisté à cet atelier qui avait pour objectif principal de favoriser l'échange d'information sur les derniers développements concernant les algues marines nuisibles dans les eaux canadiennes. Le thème principal de l'atelier de travail était "Produits nuisibles d'origine algale: impacts sur la chaîne alimentaire, les pêches et l'aquaculture". Le présent compte rendu contient: (1) les résumés des exposés oraux et des affiches qui ont été présentés à l'Atelier; (2) les six rapports des groupes de travail qui ont été mis sur pied pour discuter de l'Origine et de la Propagation des Algues Nuisibles, du Transfert des Phycotoxines dans le Réseau Trophique, de la Détection et de la Quantification des Algues Toxiques et des Toxines, et du Monitorage et de la Prédiction des Proliférations d'Algues Nuisibles; (3) un chapitre de conclusion résumant et mettant en valeur les principales recommendations des groupes de travail; et finalement en annexe, (4) deux revues résumant les programmes de monitorage et les divers travaux sur les phycotoxines qui sont effectués dans chacune des régions du MPO, accompagnées d'une liste d'hypothèses nationales sur les phycotoxines. Une liste des participants et un index des auteurs complète le compte rendu.

ABSTRACT

The Third Canadian Workshop on Harmful Marine Algae was hosted by the Department of Fisheries and Oceans, Québec region, at the Maurice Lamontagne Institute, Mont-Joli, on 12-14 May 1992. The workshop was attended by over 80 canadian and foreign participants and was aimed at a more efficient exchange of information on the latest developments concerning the subject of marine harmful algae in canadian waters. The main focus of the Workshop was "Harmful Algal Products: Impacts on the Food Web, Fisheries and Aquaculture". The present proceedings contain: (1) the abstracts of the oral and poster presentations; (2) the six reports from the Working Groups that were set up for general discussion on the following subjects: Origin and Propagation of Harmful Algae, Transfer of Phycotoxins in the Food Web, Detection and Quantification of Toxic Algae and Toxins, and Monitoring and Prediction of Harmful Events; (3) a conclusion chapter summarizing and emphasizing the most important recommendations from the Working Groups; and finally in annex, (4) two reviews highlighting the monitoring and phycotoxin programs in each DFO region, accompanied by a listing of National Working Hypotheses. A list of participants and an index of authors complete the proceedings.

INTRODUCTION

The subject of harmful and toxic marine algae has recently gained a growing scientific and public interest, both in Canada and abroad. There are many reasons for this renewed interest, but (1) the worldwide recognition of increased incidence of Paralytic Shellfish Poisoning (PSP), (2) the recent discovery of Amnesic Shellfish Poisoning (ASP) on both the east and west coasts of North America, and (3) the acute realisation of the potential risk of spreading harmful algal species and other exotic marine plant or animal species by ballast waters from ships and by other human-associated activities are certainly important causes of heightened concern.

In 1987, responding to the Prince Edward Island domoic acid crisis, the Department of Fisheries and Oceans (DFO) has increased and/or set up new research and monitoring programs, particularly, in the Scotia Fundy, Gulf and Québec regions, and has established the Phycotoxin Working Group (PWG), which is an *had hoc* national body in charge of the general coordination of marine phycotoxin research Canada-wide. This group, which has mainly a consulting function for DFO Headquarters, has rapidly identified a clear need to foster collaborative research on harmful marine algae and to encourage new research and monitoring initiatives throughout Canada.

One way the PWG uses to promote the exchange of new and unpublished scientific information on harmful marine algae and of their effects in the environment, is by sponsoring a series of National Workshops from which expert' opinions and research recommendations are sought. The First Canadian Workshop on Harmful Marine Algae was held at the Gulf Fisheries Centre in Moncton, New Brunswick, in September 1989. The general focus of this workshop was "Marine Phycotoxins in Canadian Waters: their Production and Fate". This workshop brought together representatives from government, universities and industry in both Canada and USA. The format consisted of oral presentations followed by a plenary discussion session involving all of the participants. The proceedings of this workshop, edited by Steve Bates and Jean Worms, were published in the series of Canadian Technical Reports of Fisheries and Aquatic Sciences (1989). The Second Canadian Workshop on Harmful Marine Algae was held at the Bedford Institute of Oceanography, Nova Scotia, in October 1990. Based on the previous Moncton experience, changes were introduced in the format of the Second Workshop by soliciting poster papers, by increasing the time allocated for discussion and by devoting a half day period for two large group discussions. The proceedings of this Workshop were edited by Don Gordon and published again in the series of Canadian Technical Reports of Fisheries and Aquatic Sciences (1990).

The present workshop represents, therefore, the Third Canadian Workshop on Harmful Marine Algae. It was attended by over 80 participants from government, universities and industry in both Canada and USA and, for the first time, by participants from Europe. Building upon the experiences of the other two Workshops, the Scientific

Committee of the Third Canadian Workshop has also innovated by promoting discussion into several smaller Working Groups on different topics. Specific questions were assigned to each working group on the basis of potential national interest.

Another way that the PWG uses to accomplish its mandate is by preparing annual summaries of DFO research activities on harmful marine algae using the combined resources in the different DFO regions. In 1992, the PWG enlarged the format of its annual report by including a series of program highlights, national working hypotheses for phycotoxin research, and a complete list of relevant publications by DFO staff between 1986 to 1992. These documents were distributed to the participants of the Mont-Joli Workshop at the time of registration and are included in annex with the Proceedings of the Third Canadian Workshop.

This Report contains: (1) the abstracts of all oral and poster presentations; (2) the reports from the Working Groups that were set up for discussion on the Origin and Propagation of Harmful Algae, the Transfer of Phycotoxins in the Food Web, the Detection and Quantification of Toxic Algae and Toxins, and the Monitoring and Prediction of Harmful Events; (3) a conclusion chapter summarizing and emphasizing the most important recommendations from the Working Groups; and finally in annex, (4) two reviews highlighting the monitoring and phycotoxin research programs in each DFO region, with a listing of National Working Hypotheses. A list of participants and an index of authors complete the Preceedings.

To conclude, it should be emphasized that even if this series of Canadian Workshops addresses mainly problems of specialists working on the general subject of harmful marine algae, the main objective which has motivated the organisation of these Canadian Workshops is, nevertheless, the enhancement of public awareness and protection. This is one avenue DFO scientists can use to better serve their clients.

References

Bates, S.S. and J. Worms (eds). 1989. Proceedings of the First Canadian Workshop on Harmful Marine Algae. Gulf Fisheries Centre, Moncton, N.B., September 27-28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. No. 1712, 64 p.

Gordon, C.D. Jr. (ed.). 1990. Proceedings of the Second Canadian Workshop on Harmful Marine Algae. Bedford Institute of Oceanography, Dartmouth, N.S., October 2-4, 1990. Can. Tech. Rep. Fish. Aquat. Sci. No. 1799, 66 p.

CÉDULE DU PROGRAMME
/
PROGRAM SCHEDULE

Date	Heure/hour	
12 mai	09:30	Inscription/registration Mise en place des affiches/Posters set up
	10:00	Visite de l'Institut Maurice-Lamontagne (optionnelle)/Visit of the Maurice Lamontagne Institute (optional)
	13:00	Mot de bienvenue/Opening address
		Président/Chairperson: J.-C. Therriault
	14:00	JELLETT, J.F., L.M. MARKS, J.E. STEWART, W. WATSON-WRIGHT AND M.L. DOREY. A comparison of the modified mouse neuroblastoma cell bioassay for paralytic shellfish poison (saxitoxin) with the standard AOAC mouse bioassay.
	14:20	LAYCOCK, M.V., P. THIBAULT, S.W. AYER, J.A. WALTER AND J.L.C. WRIGHT. Purification and characterization of paralytic shellfish poisons from Gaspé strains of *Alexandrium excavatum*.
	14:40	TODD, E.C.D., J.M. MacKENZIE, C.F.B. HOLMES AND D.L. PARK. The mouse bioassay, protein phosphatase inhibition bioassay, and the solid phase immunobead assay for testing tropical fish for possible ciguatoxin.
	15:00	WILDISH, D.J., J.L. MARTIN, AND M. RINGUETTE. Methods to assess potentially harmful microalgae in the Bay of Fundy salmonid culture industry.
	15:20	JELLETT, J.F. AND J.E. STEWART. The role of bacteria in the growth and production of paralytic shellfish poisons by the toxic dinoflagellate *Alexandrium tamarense*.

DATE	**Heure/hour**	
	15:40	**Session d'affichage #1/Poster session #1** Pause café/Coffee break
	16:15	**Ateliers de travail session #1/Working groups session #1**
	18:00	Rencontre sociale/Social mixer
	19:30	Départ de l'autobus pour les hôtels/Bus departure for hotels
13 mai		Président/Chairperson: **Serge Demers**
	08:40	**BATES, S.S.** AND C. LÉGER. Response of *Nitzschia pungens f. multiseries* to irradiance: growth and domoic acid production.
	09:00	**DOUGLAS, D.J.** AND S.S. BATES. Domoic acid production by an axenic culture of *Nitzschia pungens* forma *multiseries*.
	09:20	**ROELKE, D.L.**, M.C. VILLAC, G.A. FRYXELL, R.D. VAN PUTTE, K.R. BUCK, F.P. CHAVEZ. *Pseudonitzschia australis* frenguelli from Monterey Bay, California: toxicity in the Bay and culture experiments.
	09:40	**WRIGHT, J.L.C.**, D.J. DOUGLAS, U.P. RAMSEY AND J.A. WALTER. Biosynthesis of domoic acid by the marine diatom *Nitzschia pungens* forma *multiseries*, determined with (^{13}C)-labelled precursors and nuclear magnetic resonance.
	10:00	**BLASCO, D.** What needs to be measured to predict *Alexandrium excavatum* blooms?
	10:20	**Session d'affichage #2/Poster session #2** Pause café/coffee break
	11:00	Ateliers de travail session #2 /Working groups session #2
	12:30	Dîner/Lunch

Date	Heure/hour	
		Président/Chairperson : **Maurice Levasseur**
	13:30	SCHWINGHAMER, P., **M. HAWRYLUK**, C. POWELL AND C.H. MacKENZIE. Winter occurrence of PSP in inshore Newfoundland waters caused by resuspended hypnozygotes of *Alexandrium fundyense.*
	13:50	**ROY, S.** Contrôle de la germination des kystes d'*Alexandrium sp.* récoltés au large de Baie-Comeau.
	14:10	**MARTIN, C.**, J.A. FOX AND K. LILJESTRAND. Recent observations on paralytic toxins in the Atlantic deepsea scallop, *Placopecten Magellanicus*, in U.S. and Canadian waters.
	14:30	**TURRIFF, N.**, J.A. RUNGE AND A.D. CEMBELLA. Feeding and toxin accumulation behavior of the copepod *Calanus finmarchicus* in the presence of the red-tide dinoflagellate *Alexandrium excavatum.*
	14:50	**CATTETT, M.** AND J.R. GERACI. Distribution and elimination of ingested brevetoxin (PbTx-3) in rats.
	15:10	**CHEBIB, H.**, A.D. CEMBELLA, AND P.D. ANDERSON. Cinétiques d'accumulation et d'élimination de toxines paralysantes (IMP) de deux populations de moules *Mytilus edulis* transplantées et exposées à des blooms naturels d'*Alexandrium excavatum*.
	15:30	OUTERBRIDGE, G., A.M. RENATA AND **D.J. SCARRATT**. Uptake and elimination of domoic acid by mussels (*Mytilus sp.*) in various experimental conditions.
	15:50	**Session d'affichage #3/Poster session #3** Pause café/Coffee break/
	16:20	**Ateliers de travail session #3/Working groups session #3**
	18:30	Départ de l'autobus pour le restaurant/ Bus departure for restaurant

Date **Heure/hour**

14 mai Président/Chairperson: **Serge Demers**

08:40 **LEVASSEUR, M., M.D. KELLER, E. BONNEAU, D. D'AMOURS AND W.D. BELLOWS.** Toward an understanding of the oceanographic basis of a cod fishery problem: blackberry feed.

09:00 **WRIGHT, J.L.C., D.J. DOUGLAS, U.P.RAMSEY AND J.A. WALTER.** Domoic acid on the West coast of America. A brief report and overview.

09:20 **G. BUGDEN, G., R. FORBES, D.C. GORDON, B. HUPPERTZ, P.D. KEIZER, M. LEVASSEUR, J.L. MARTIN, R. PENNEY, J. SMITH, D.V. SUBBA RAO, D.J. WILDISH AND P. YEATS.** Overview of Canadian phytoplankton monitoring programs.

09:40 **MARTIN, J.L. AND D.J. WILDISH.** Integrated water column versus Niskin bottle sampling in the southwest Bay of Fundy.

10:15 **Session plénière/Plenary session**

12:30 Fin de l'atelier/End of workshop

RÉSUMÉ DES PRÉSENTATIONS ORALES

/

ABSTRACT OF ORAL PRESENTATIONS

WHAT NEEDS TO BE MEASURED TO PREDICT *ALEXANDRIUM EXCAVATUM* BLOOMS?

Blasco, D.

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The results of principal component analysis of taxonomic phytoplankton data collected in many different oceanic regions show always a segregation of the species into the major taxonomic groups: diatoms, coccolithophorids and dinoflagellates. The universality of these findings suggest strongly that all the species within a group have a common response to certain environmental conditions. Furthermore, analysis of phytoplankton data of the same region but in different years, shows that although the dominant species within a group often change, the "groups" as such reappear if the same environmental conditions reoccur. The implications of these findings in the prediction of toxic dinoflagellates blooms (i.e. *A. excavatum*), and in the designing of future field and laboratory experiments will be discussed.

RESPONSE OF *NITZSCHIA PUNGENS F. MULTISERIES* TO IRRADIANCE: GROWTH AND DOMOIC ACID PRODUCTION

Bates, S.S. and C. Léger

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The domoic-acid-producing diatom *Nitzschia pungens f. multiseries* (clone POM) was grown in semi-continuous culture at 16 irradiance levels (10 to 250 µEinst $m^{-2}s^{-1}$) for 33 days. Cultures were diluted with fresh medium prior to reaching the stationary phase so that 4 exponential growth curves were obtained for each irradiance level during this period. No evident light limitation of division rate was observed for the cultures grown at 30 to 250 µEinst $m^{-2}s^{-1}$. The culture grown at 10 µEinst $m^{-2}s^{-1}$ has a lower division rate than the other, but only one growth cycle was obtained because of the low division rate. Cultures from the above experiment were conditioned for a further 3 weeks at 35, 90, 130, and 200 µEinst $m^{-2}s^{-1}$ prior to a second experiment. Division rates were not significantly different at the 3 highest irradiance levels, but a slightly lower division rate was observed at 35 µEinst $m^{-2}s^{-1}$, suggesting the beginning of light limitation. Rates of domoic acid production and values of domoic acid per cell

were likewise not significantly different at the 3 highest irradiance levels. Cells grown at 35 µEinst m^{-2}s^{-1} produced significantly lower levels of domoic acid and at a lower rate than those grown at the higher irradiance levels. There was an inverse correlation between chlorophyll *a* per cell and growth irradiance, as expected. These results show that there is an uncoupling between growth and domoic acid production at a threshold irradiance level between 35 and 90 µEinst m^{-2}s^{-1}, and suggest that the division rate of *N. pungens* is not limited until at least 35 µEinst m^{-2}s^{-1}. In culture experiments, *N. pungens* should be grown at an irradiance level ≥ 100 µEinst m^{-2}s^{-1} in order to avoid light limitation of domoic acid production.

DISTRIBUTION AND ELIMINATION OF INGESTED BREVETOXIN (PbTx-3) IN RATS

Cattet, M. and J.R. Geraci

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Among the ubiquitous marine dinoflagellates in North America is *Gymnodinium breve* whose toxin provokes mass mortalities of fishes (Steidinger *et al.* 1973), and causes neurologic shellfish poisoning in humans. We have discovered that this toxin can be carried through the food chain, and thereby represents a threat to marine mammals. Brevetoxin (PbTx) carried by menhaden was incriminated in the death of at least 750 bottlenose dolphins from New Jersey to Florida (Geraci 1989), and through tunicates in the poisoning of manatees in Florida (O'Shea *et al.* 1991). As a first step towards understanding the pathologic effects of long-term ingestion of brevetoxin (PbTx), we determined how $[^{3}H]$PbTx-3 was distributed, stored, and eliminated when ingested at low dosages (10 µg/kg) by Fisher 344 rats. To relate our findings to previous studies, some rats received an equivalent dosage of toxin intravenously. Following its ingestion, PbTx-3 concentrated primarily in liver and spleen. We believe toxin metabolites have affinity for these organs, making them potential targets for toxic damage. Brevetoxin also concentrated in stomach and intestines as a natural consequence of ingestion. Proportionally more ingested toxin was cleared through the urine than the faeces, emphasizing the vital role of the kidneys in the excretion of PbTx-3, as well as their vulnerability as a potential site of toxic action. Future studies will examine how accumulating toxin affects organ function.

CINÉTIQUES D'ACCUMULATION ET D'ÉLIMINATION DE TOXINES PARALYSANTES (IMP) DE DEUX POPULATIONS DE MOULES *MYTILUS EDULIS* TRANSPLANTÉES ET EXPOSÉES À DES BLOOMS NATURELS D'*ALEXANDRIUM EXCAVATUM*

Chebib[1], H.A., A.D. Cembella[2] and P.D. Anderson[3]

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Deux populations isolées de moule bleue *Mytilus edulis* ayant hérité de passés différents d'expositions aux toxines paralysantes furent exposées à des blooms naturels d'*Alexandrium excavatum* qui l'espèce responsable pour l'intoxication paralysante par les mollusques (IPM), lors d'une expérience de transplantation. Des moules prevenant d'un environnement exempt d'algues toxiques (Iles-de-la-Madeleine, Québec; MAD) furent transplantées dans des case *in situ* et suspendues dans la colonne d'eau à une station située dans le bas St-Laurent, parallèlement avec des moules provenant d'une zone d'intoxication chronique (Cap Chat, Québec; CAP). Les moules des deux groupes furent maintenues au site expérimental du début de l'été, avant l'initiation du bloom estival d'*Alexandrium*, jusqu'à tard en automne, lorsque la colonne d'eau était exempte de cellules d'*Alexandrium* et que les niveaux de toxines dans les moules se rapprochèrent de la limite réglementaire de consommation humaine (80 µg STXeq 100g^{-1} portion comestible). Durant cette période, les moules furent exposées à deux blooms consécutifs d'*Alexandrium excavatum* qui différaient dans leurs durée et concentration cellulaire maximale. Une analyse de discrimination multivariée fut réalisée pour distinguer les profils différents d'accumulation et d'élimination de toxines pour les deux populations. Les concentrations de toxines furent déterminées par chromatographie liquide à haute performance (CLHP) des composants toxiques. La toxicité moyenne des cellules d'*Alexandrium* se trouvait à un niveau de 3.3 pg STXeq/cellule durant le premier bloom, et à un niveau plus élevé de 13.8 pg STXeq/cellule durant le second bloom. Les toxines N-sulfo-carbomoyl C_2 (60.0%) et les carbamates neosaxitoxine (neoSTX) (29.3%) et saxitoxine (STX) (4.3%) dominèrent sur une base molaire. Le niveau d'accumulation de toxines à 2.2 x 10^3 nmol/g pour les moules de MAD était moins élevé que les moules de CAP qui affichaient un niveau maximal d'une concentration de 4.2 x 10^3 nmol/g. Cependant, l'accumulation de toxines à 2.2 x 10^3 nmol/g pour les moules de MAD était moins élevé que les moules de CAP qui affichaient un niveau maximal de concentration de 4.2 x 10^3 nmol/g. L'accumulation maximale de toxine durant le second bloom était presque identique pour les deux groupes. Les toxines présentes dans les deux groupes de moules étaient par ordre décroissant d'importance C_2, neoSTX, STX et les gonyautoxines 1 et 4 (GTX_1, GTX_4), dans les deux groupes de moules. Des biotransformations de toxines furent observées dans les changements des rapports entre les paires épimériques dans les phases d'accumulation et d'élimination de toxines des deux blooms. Ces observations ont démontré une équilibration significative de GTX à GTX_1, de GTX_3 à GTX_2 et une baisse dans les rapports de neoSTX à STX durant la première phase d'accumulation de toxine des moules de CAP et MAD. Seul le quotient de neoSTX:STX changea durant la phase cumulative du second bloom où les concentrations relatives de STX augmentaient constamment relativement aux concentrations de neoSTX. Les observations ont démontré également que des différences significatives furent observées entre les rapports de GTX_4 à GTX_1 dans les moules de MAD et de CAP pour les deux blooms et dans les proportions de GTX_3 et GTX_2 dans la phase de désintoxication du premier bloom. Les niveaux de toxines des deux populations parvinrent à près de 10% de leur concentration maximale en 14 et 19 jours à la suite des premiers et seconds blooms, respectivement. Suivant le premier bloom, les demi-vies des toxines dans les moules de CAP et de MAD étaient de 4.0 jours et de 2.2 jours, respectivement. Les moules importées de CAP transférèrent plus de toxines aux tissus (23% nmol/g et 18% µg STXeq/100g) que les moules de MAD qui en transférèrent seulement 15% en terme de nmol/g et 18% µg STXeq/100g seulement. Il semblerait que les moules de cultures accumulent et se débarassent des toxines de manière plus efficace que les moules préalablement exposées aux PSP, ce qui les rendraient moins dangereuses à la consommation suite à un bloom de phytoplancton toxique. Cette recherche est unique dans son genre car elle décrit les réactions des moules dans un environnement naturel et non dans un cadre contrôlé en laboratoire.

DOMOIC ACID PRODUCTION BY AN AXENIC CULTURE OF *NITZSCHIA PUNGENS* F. *MULTISERIES*

Douglas[1], **D.J. and S.S. Bates**[2]

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A microbially-contaminated culture of *Nitzschia pungens* f. *multiseries* was treated with gentaminin followed by a combination of penicillin and streptomycin. An axenic culture was successfully isolated as evidenced by sterility tests and examination using epifluorescent microscopy. This isolate was grown in a 12-L fermentor which was modified to include supply and measurement of light for photosynthesis. The axenic culture began producing domoic acid (DA) at the end of exponential growth. Domoic acid was produced during both the lag and stationary phases, when cell division rates were low or zero. The rate of production reached 0.5 pg DA.cell^{-1}.day^{-1} two to three days after the onset of stationary phase. These results provide the first evidence that *N. pungens* f. *multiseries* produces DA in the absence of other microorganisms. Preliminary experiments comparing axenic strains with microbially-contaminated cultures indicate that the non-axenic strains may survive longer in stationary phase and may produce substantially greater amounts of DA. Whether these observed differences reflect deletirous effects of treatment with antibiotics or represent evidence of bacterial mechanism(s) for enhancement of cell survival and DA production is currently under investigation.

WINTER OCCURRENCE OF PSP IN INSHORE NEWFOUNDLAND WATERS IS CAUSED BY RESUSPENDED HYPNOZYGOTES OF *ALEXANDRIUM FUNDYENSE*

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The growth of the aquaculture industry in Newfoundland, especially along the northeast coast, has focussed attention on the recent incidence there of paralytic shellfish poisoning (PSP) intoxication of shellfish, especially mussels and scallops. Science Branch of DFO in Newfoundland has collected water, sediment, and stomach contents on an opportunistic basis to determine the factors which lead to elevated levels of PSP toxins in the shellfish assayed by Inspection Services Branch, DFO, St.John's. The sampling began in 1989 with a province-wide survey of inshore sediments near mussel farms or collection sites for wild populations. Our samples indicated a possible relationship between the occurrence of sediment resting cysts, or hypnozygotes of *Alexandrium* in the sediments and the recorded incidence of PSP. In the winter of 1991-1992, we examined the stomach content of mussels and scallops from embayments in Notre Dame Bay on the northeast coast of Newfoundland. The animals contained a wide range of toxin levels which were very closely related to the numbers of cysts in the stomach. Water samples taken at the same sites did not contain any planktonic *Alexandrium*, and nor did the mussel stomachs. A sudden increase in toxin levels in mussels from some sites occurred after several days of strong northeasterly winds and levels remained high during at least a month of sustained westerly winds. Hypnozygotes in wind-resuspended sediments

were definitely the agents of intoxication of suspended mussel cultures at these sites. Other environmental factors influenced the effect of wind to resuspend the sediment resting cysts in numbers high enough to intoxicate the mussels at some site and not others. Bottom type appears to be especially important.

A COMPARISON OF THE MODIFIED MOUSE NEUROBLASTOMA CELL BIOASSAY FOR PARALYTIC SHELLFISH POISON (SAXITOXIN) WITH THE STANDARD AOAC MOUSE BIOASSAY

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The mouse neuroblastoma cell bioassay, developed by Kogure and colleagues, has been modified so that determinations of saxitoxin equivalents (STC eq.) can be made with a microplate reader. This eliminates the need to count individual cells and provides a method suitable for routine research and regulatory work. The lower detection limit of the cell bioassay is 10 ng STX per ml of extract (= 2.0 µg/100 g shellfish tissue) compared to 200 ng/ml (= 40 µg/100 g) for the mouse bioassay. The precision of the two bioassay methods is similar with most samples reliable to within = 20%. The cell bioassay has been used routinely to determine STX eq. in acid extracts of culture of *Alexandrium excavata* and *A. fundyense*. Comparisons of the two bioassays using acid extracts of dinoflagellates and AOAC extracts of shellfish tissues showed that the tissue culture bioassay provides results which are virtually identical to those obtained with the mouse bioassay ($r^2 > 0.95$). Interfering substances in low toxicity extracts could be removed with an SPE-C18 cleanup.

THE ROLE OF BACTERIA IN THE GROWTH AND PRODUCTION OF PARALYTIC SHELLFISH POISONS BY THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE*

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Axenic and non-axenic cultures of *Alexandrium tamarense* were grown in a modified F-medium made with artificial seawater at salinities representing estuarine and open ocean conditions. Toxin production by the dinoflagellates was measured using the modified neuroblastoma cell bioassay. Disappearance of inorganic phosphate, and nitrate and nitrite from the medium was measured, along with cell growth. The apparent role of bacteria in the dynamics of toxic blooms of *Alexandrium tamarense* will be discussed.

OVERVIEW OF CANADIAN PHYTOPLANKTON MONITORING PROGRAMS

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At the present time, it is known that approximately ten species of marine algae occurring in Canadian marine waters have the potential to cause harmful effects on both marine organisms and human consumers. During the domoic acid crisis in eastern Prince Edward Island in late 1987, it became apparent that little is known about the

species of phytoplankton that are normally present in Atlantic coastal waters. Therefore an expanded phytoplankton monitoring program was established throughout the Atlantic zone including the Department's Scotia-Fundy, Gulf and Quebec Regions. The 3-year program would determine in what areas and at what time phycotoxins were likely to be present. The resulting background information that would be collected would provide a basis for understanding whether these toxic events are normal or are related to exceptional meteorological events or anthropogenic activity. An overview is presented of the phytoplankton monitoring program activities in each Region. The program evolved somewhat differently in each Region as a function of the particular resources available and the demands of local industry. The resulting databases provide a unique opportunity for the analysis of the frequency of occurrence and spatial distribution of phytoplankton species over a large area for a 3 year period (see complete report in annex 1.)

PURIFICATION AND CHARACTERIZATION OF PARALYTIC SHELLFISH POISONS FROM GASPÉ STRAINS OF *ALEXANDRIUM EXCAVATUM*

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The development of new methods of analysis for PSP's has been hampered by limited availability of pure compounds. Up to twenty-four derivatives of saxitoxin are known, although about half this number have been reported from natural sources. Saxitoxin has been commercially available, but it is not the most common PSP in algae or shellfish, at least on the east coast of North America. The C toxins (disulfated derivatives) were dominant in the strains of *Alexandrium* that we have examined and GTX's 2 and 3 (monosulfated derivatives) in scallop. Response factors for PSP's can differ considerably in different analytical methods and reliable standards of known purity and toxin concentration of several PSP's are urgently needed. We have obtained pu saxitoxin, neosaxitoxin, GTX's 2 and 3 and C toxins 1 and 2 from cultures of toxic phytoplankton and synthesized two derivatives, decarbamoylsaxitoxin and 11-hydroxysaxitoxin, in sufficient quantities to prepare calibration solutions for the separate toxins. The dinoflagellate, *Alexandrium excavatum* Gaspé strain PR 103F, was chosen for culture because of the unusually large amounts of saxitoxin and neosaxitoxin that are produced. A typical extract of cells (50 g wet weight), contained 93 mg total PSP's and, after purification on Biogel P-2 and Biorex-70 columns, yielded saxitoxin (27 mg), neosaxitoxin (11 mg), and C toxins 2 and 3 (38 mg). GTX 2 and 3 were prepared by mild acid hydrolysis of the C toxins. Ion spray mass spectrometry, capillary electrophoresis and an HPLC-fluorescence method were used to monitor purity and for quantitative analysis based on response factors. Absolute amounts of pure toxins were obtained from proton NMR spectral data calibrated against sucrose solution.

TOWARD AN UNDERSTANDING OF THE OCEANOGRAPHIC BASIS OF A COD FISHERY PROBLEM: BLACKBERRY FEED

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Atlantic cod (*Gadus morhua*) caught between June and August along the Labrador and Newfoundland coasts and in the Strait of Belle-Isle often have a strong smell of sulphur. This problem, locally called "blackberry feed", lasts 2-3 weeks and may represent a financial lost for the fishermen. Early observations have shown that the sulphur smelling cod had fed almost exclusively on *Limacina helicina*, an herbivorous pteropod, typical of the cold waters of the Labrador Current. Chemical analysis of gut contents revealed that dimethylsulfide (DMS), a non-toxic molecule of algal origin, was responsible for the odour. In August 1991, we conducted a cruise in the northeastern Gulf of St. Lawrence and in the Strait of Bell-Isle in order to confirm the algal origin of DMS and to determine the chemical, biological and physical factors responsible for DMS production and transfer in the food web. During this cruise, concentrations of dimethylsulfoniopropionate (DMSP; the organic molecule precursor of DMS) were maximum in the Gulf and minimum in the Labrador current waters. A significant correlation was found between particular DMSP and the abundance of the Prymnesiophyceae (mostly *Emiliana huxleyi* and *Chrysochromulina spp.*), a group of algae known to produce DMSP (*L. helicina*) was present in the Gulf and in the Strait, but individuals with diameters greater than 2 mm, such as those normally found in cods, were captured only in the DMSP-poor waters of the Labrador current. However, the DMSP content of *L. helicina* was always high (0.4 to 2.4 mg DMSP/g *L. helicina*) and sufficient to create the blackberry feed symptoms. A significant linear relationship was found between the concentration of DMSP in *L. helicina* and in the particular matter. Bioassay experiments suggest a slow turnover rate of DMSP in *L. helicina* which consequently may accumulate high internal concentrations of DMSP even in DMSP-poor waters. This may explain the recurring nature of the blackberry feed problem in the Canadian East Coast waters.

RECENT OBSERVATIONS ON PARALYTIC TOXINS IN THE ATLANTIC DEEPSEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, IN U.S. AND CANADIAN WATERS

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Atlantic deepsea scallops, *Placopecten magellanicus* Gmelin were collected from more than 600 stations in 1990 and 1991 from North Carolina to Nova Scotia. Approximately half of the stations collected lay east of 70 degrees west longitude (Nantucket Shoals and Georges Bank including the Canadian sector). Whole animal analysis of these samples is ongoing. Using high performance liquid chromatography with fluorescence analysis and mouse bioassay, we have documented widespread occurrence of paralytic toxins in animals on Georges Bank. Bioassays and some limited HPLC determinations reveal little or no toxicity west of this region. Geographic distribution of toxicity on the banks is highly variable while toxin profiles reveal the consistent presence of saxitoxin and some of its derivatives. Toxicity is consistent with an *Alexandrium* source even though the occurrence of this dinoflagellate has not been, so far, observed in large numbers in this offshore area.

INTEGRATED WATER COLUMN VERSUS NISKIN BOTTLE SAMPLING IN THE SOUTHWEST BAY OF FUNDY

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Many regions of the world have altered their sampling technique for phytoplankton distribution and abundance from a closed bottle sampling (such as Niskin bottles) to an integrated water column sampler that takes a profile of the water column. This allows all the upper water column to be sampled so that flagellates that can concentrate in layers under stratified conditions are not missed. Results from a comparison study conducted in the southwest Bay of Fundy between an integrated sampler and the deployment of Niskin bottles at varying depths are presented. Water samples were collected at various locations throughout the southwest Bay of Fundy. An integrated water sampler was fabricated to collect water from: the upper 3 m, the

next 5 m and the lower 5 m. Samples collected from the integrated sampler were compared with those from a bucket at the surface and Niskin bottles at 5 m and one water above bottom. Due to the additional time required to collect and analyze samples, sampling was not done on a regular basis at any stations except at Deadmans Harbour. Our results indicate a statistically significant correlation between most numbers of phytoplankton observed with the two methods. On only one occasion in July, did we find an obvious difference between the two techniques when enumerating dinoflagellates. The sample collected with a bucket from the surface had up to 60% more dinoflagellates observed from the surface than the integrated sampler. For the inshore region of the southwest Bay of Fundy, the Niskin bottle is the most feasible method for sampling. The waters are well mixed with many phytoplankton populations throughout the water column. The integrated sampler was also extremely awkward to operate in these waters - with the strong winds, extreme tides, and dynamic water currents.

PSEUDONITZSCHIA AUSTRALIS FRENGUELLI FROM MONTEREY BAY, CALIFORNIA: TOXICITY IN THE BAY AND CULTURE EXPERIMENTS

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Several hundred pelican deaths in Monterey Bay, CA, during the autumn of 1991 alerted the scientific community to a toxic outbreak of domoic acid (DA). Intensified sampling revealed that toxic events were widespread along the west coast of the United States contaminating anchovies, razor clams, mussels, and birds. Toxin levels were high enough to merit temporary closure of some fisheries. *Pseudonitzschia australis*, closely related to the DA producer *Nitzschia pungens* f. *multiseries*, was dominant in the phytoplankton at the time of the outbreak, strongly implicating the diatom as another producer of DA. Plankton samples from Monterey Bay were collected and analyzed for DA concentrations in mid-October, late-October, and mid-November, yielding DA-positive results. These dates correspond with high anchovy toxicity in the Bay. Indication that the DA detected in the net hauls came from within the *P. australis* cells was that the DA concentration per ml of net haul positively correlated with the cell concentration of *P. australis* per ml of net haul ($r = 0.54$ mid-Oct., $r = 0.84$ late-Oct., $r = 0.76$ mid-Nov.). The net hauls show a decrease in DA concentration per cell from an average of 17.25 pg/cell in mid-Oct. to 7.92 pg/cell in mid-Nov. Isolations from a toxic net haul have produced viable clonal cultures of *P. australis*. To test the hypothesis that *P. australis* produces DA, a culture (MB1c) was grown up in Guillard's f/2 media, and sampled every third day for DA analysis. The temperature and salinity remained constant at 15 and 33 °C, respectively. Light was on 24 hrs/day at a level of 124.5 µEinst.m^{2}s. *P. australis* did not produce DA under our conditions over a 34 day period. Our detection threshold based on the culture at 34 days was 0.02 pg/cell. Major nutrient levels along with chlorophyll concentration were also sampled during the growth period and will be reported. If *P. australis* produces DA it does not appear to follow the same pattern as *N. pungens* f. *multiseries*. The conditions under which DA might be produced in *P. australis* must be determined before a monitoring program can be used as a reliable warning to fisheries managers.

CONTRÔLE DE LA GERMINATION DES KYSTES D'*ALEXANDRIUM SP.* RÉCOLTÉS AU LARGE DE BAIE-COMEAU

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L'hypothèse du contrôle de la germination des kystes d'*Alexandrium sp.* par la température et la lumière a été testée en laboratoire sur des kystes provenant de sédiments côtiers de la région de Baie-Comeau (rive nord de l'estuaire maritime du Saint-Laurent). Les résultats indiquent que les facteurs testés ont très peu d'influence sur l'exkystement de cette algue toxique et que la période de l'année pendant laquelle sont effectués ces tests peut affecter les résultats. Ces informations seront discutées en considérant une autre hypothèse concernant le contrôle de la germination de ces kystes: celle d'un rythme endogène, propre au cycle de vie de cette espèce d'algue.

UPTAKE AND ELIMINATION OF DOMOIC ACID BY MUSSELS (*MYTILUS SP.*) IN VARIOUS EXPERIMENTAL CONDITIONS

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Feeding *Mytilus edulis* on both toxic and non-toxic *Nitzschia pungens* reveals no evidence that the toxin stimulates the feeding or ingestion rate of mussels. Commercial size mussels can be induced to accumulate up to at least 66.5 µg of domoic acid g^{-1} of digestive gland when maintained for 144 hours in laboratory conditions and fed domoic acid producing strains of *N. pungens*. Variables such as water temperature, salinity, prior starvation, concentration of toxic algae and duration of exposure may affect the accumulation rate. The domoic acid content of both laboratory and naturally contaminated mussels can be reduced to acceptable levels by holding mussels in flowing seawater, or in a recirculating system equipped with UV sterilisation, the latter option being recommended for commercial applications. A time frame of less than 48 hours is envisaged for mussels containing up to 100 µg domoic acid g^{-1} of digestive gland.

THE MOUSE BIOASSAY, PROTEIN PHOSPHATASE INHIBITION BIOASSAY, AND THE SOLID IMMUNOBEAD ASSAY FOR TESTING TROPICAL FISH FOR POSSIBLE CIGUATOXIN

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Organic solvent extracts of tropical fish obtained in Toronto were analyzed by the mouse bioassay, the protein phosphatase inhibition bioassay and the solid phase immunobead assay (Ciguatect™). From approximately the same amounts of fish (200

g) varying amounts of extracts were received (515-9440 mg). Eighty-five percent of the extracts caused mouse deaths within 24 h, 6% of extracts showed some symptoms and only 9% gave no symptoms. These same extracts showed protein phosphatase-1 inhibition (PPI) to > 70 μg okadaic acid equivalents (OA eq)/100 g (31% of extracts), between 1 and 69 μg OA eq/100 g (55%), and < 1 μg OA/eq)/100g (14%). The toxic compounds in the fish, however, appear to be distinct from OA or dinophysis toxin-1. When the same fish were tested with the Ciguatect™ test kit, 46% of them reacted moderately well, 9% weakly and 45% negatively. Although each of these methods separately showed toxic potential in some of the market fish, there was no apparent correlation between the results. The results support the theory that there are several toxins associated with ciguatera since PP1 measures the OA suite of toxins, the immunobead dipstick detects polyether compounds including OA and ciguatoxin, and the mouse bioassay responds to toxins active on IP injection. The next task is to try and identify each type of toxin found in the fish.

FEEDING AND TOXIN ACCUMULATION BEHAVIOR OF THE COPEPOD *CALANUS FINMARCHICUS* IN THE PRESENCE OF THE RED-TIDE DINOFLAGELLATE *ALEXANDRIUM EXCAVATUM*

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The planktonic copepod *Calanus finmarchicus* is a dominant component of the zooplankton in the St. Lawrence estuary. Feeding rates of female *Calanus* on three isolates of *Alexandrium excavatum* of varying toxicities were measured. Clearance rates on non-toxic *Alexandrium* were on the order of 5 ml.i^{-1}.h^{-1}, but were near-zero in the presence of either toxic strain. These results indicate that *C. finmarchicus* was capable of detecting toxicity in *Alexandrium* and of altering its feeding behaviour accordingly. Analysis by high-performance liquid chromatography with fluorescence detection revealed the presence of paralytic shellfish poisoning (PSP) toxins in the tissues of the copepods. The evidence suggests that *C. finmarchicus* initially ingests the cells and accumulates the toxins in the body. Blooms of toxic *Alexandrium excavatum* occur annually in the lower St. Lawrence estuary and thus could influence the population dynamics of *C. finmarchicus* in the region. *Calanus finmarchicus* could be an important vector in the transmission of toxins through the food web.

METHODS TO ASSESS POTENTIALLY HARMFUL MICROALGAE IN THE BAY OF FUNDY SALMONIC CULTURE INDUSTRY

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Since 1972, the salmonic culture industry in the SW Bay of Fundy has become the most important of those producing fish or shellfish in this area, with a 1990 value of $74.25 million. During this period, the industry has experienced no mortalities attributable to harmful marine microalgae. Yet, most other places where a salmonid culture is established, e.g. Norway, Scotland, Iceland, Chile, Pacific Canada and New Zealand, have experienced significant fish kills due to marine microalgae. Because of the high probability of similar fish kills re-occurring, we are developing ecologically relevant bioassays to assess potential microalgal problems which could take place in the Bay of Fundy. Behavioral bioassays are with single, or groups, of smelts in a tank which is observed from above with a video camera. Locomotory behavior is analyzed by available PC software as speed, distance travelled, avoidance, etc., in a control and treatment period. During treatment, the tank is dosed with a known microalgal concentration of species such as *Alexandrium fundyense*, *Nitzschia pseudodelicatissima* and *chaetoceros sp.*. Physiological bioassays include electrocardiograms (ECG) in live smelts. It should be possible to test neurotoxins and metabolic inhibitors from microalgae by this method. An acoustic tag developed by Vemco Ltd., R.R. # 4, Armdale, N.S., B3L 4J4, is surgically implanted in the body cavity, just behind the heart. An electrode from the tag is pushed through the pericardial wall and the impulse registered as an acoustic sound with the aid of a battery in the tag. The sound is monitored by a hydrophone and relayed to a receiver unit where it is digitized. Heart beat rates are then compared in a control and treatment period as with the behavioral bioassays.

BIOSYNTHESIS OF DOMOIC ACID BY THE MARINE DIATOM *NITZSCHIA PUNGENS* FORMA *MULTISERIES*, DETERMINED WITH [^{13}C]-LABELLED PRECURSORS AND NUCLEAR MAGNETIC RESONANCE

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Domoic acid is a neuroexcitatory amino acid identified as the toxin responsible for an outbreak of shellfish poisoning in eastern Canada in 1987, and recently implicated in the deaths of aquatic birds on the West Coast of the U.S.A. For the Canadian incident, it has been established that domoic acid is produced during stationary growth phase by the diatom *Nitzschia pungens* forma *multiseries*. Domoic acid belongs to a class of structurally similar metabolites called the kainoids, and although nothing was known about their biogenesis, the assembly of kainoids posed an interesting biosynthetic problem. We report the results of ^{13}C labelled precursor studies. The data reveal a new route to the assembly of a proline ring structure by condensation of an activated glutamate derivative with an isoprenol chain which suggest a common biosynthetic pathway for all the kainoids. To our knowledge this is the first time that biosynthetic experiments have been reported on a natural product from a diatom.

DOMOIC ACID ON THE WEST COAST OF AMERICA, A BRIEF REPORT AND OVERVIEW

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In the fall of last year, the mysterious deaths of penguins and cormorants in the Monterey Bay area of California was traced by IMB scientists to domoic acid intoxication following ingestion of anchovies containing the toxin. The source of domoic acid was found to be a diatom *Pseudonitzchia australis* (= *Nitzschia pseudoseriata*). Shortly after, mild domoic intoxication of humans in Washington was reported, following consumption of domoic acid-contaminated razor clams. Further investigations in Washington and Oregon revealed that mussels and oysters in the area were unaffected, but that the viscera of Dungeness crabs contained levels of domoic acid above the 20 ppm level. To date the source of this domoic acid outbreak has not been determined. Subsequent chemical analytical surveys have revealed the presence of domoic acid from southern California to Alaska. Not surprisingly, the crab and aquaculture industry is reeling from this discovery, and monitoring strategies are being developed to deal with the problem. Details of the size, scope and unique problems associated with this outbreak will be described.

RÉSUMÉ DES AFFICHES
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ABSTRACT OF POSTERS

IONSPRAY MASS SPECTROMETRY OF MARINE TOXINS: ANALYSIS OF PARALYTIC SHELLFISH POISONING TOXINS BY FLOW INJECTION, LC-MS AND CE-MS

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Ionspray mass spectrometry has been used to monitor the purification of saxitoxin, the parent compound in the family of toxins responsible for paralytic shellfish poisoning (PSP), from a strain of the dinoflagellate *Alexandrium excavatum*. Quantitative results obtained by flow injection analysis are compared to those obtained by high performance liquid chromatography with post-column oxidation and fluorescence detection. The coupling of liquid chromatography and capillary electrophoresis with ionspray mass spectrometry is described for the separation of mixtures of PSP toxins and the highly potent pufferfish toxin tetrodotoxin. Tandem mass spectrometry is used to provide structural information, and the ability to distinguish isomeric PSP toxins, both chromatographically and mass spectrometrically, is described.

DEVELOPMENT OF AN IMMUNOFLUORESCENCE METHOD TO DETECT THE DIATOMS *NITZSCHIA PUNGENS* F. *MULTISERIES* AND *NITZSCHIA PUNGENS* F. *PUNGENS*

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Polyclonal antibodies were developed to cell surface antigens of the two forms of the pennate diatom, *Nitzschia pungens*, i.e., f. *multiseries* (the domoic-acid-producing form), and f. *pungens* (the non-toxic form). Positive reactions were visualized with epifluorescence microscopy, using an indirect immunofluorescence assay. First, primary rabbit antibodies, against one form or the other of *N. pungens*, were incubated with live or preserved phytoplankton cells. Then the cells were incubated with goat anti-rabbit secondary antibodies conjugated to the immunofluorescent label, florescein isothicyanate (FITC). Seven clones of f. *multiseries*, 5 clones of f. *pungens*, 6 unidentified *Nitzschia spp.*, and 6 other phytoplankton species have so far been examined. Positive reactions of f. *pungens* antisera with f. *pungens* were observed at a titre of 1:500, but a working dilution of 1:20 was used to maximize the intensity of labelling. The antiserum against f. *multiseries*, obtained from only 1 rabbit, exhibited weak positive reactions at a titre of 1:10 and was eventually dropped from the study; additional rabbits have been immunized with f. *multiseries*. No differences in reactivity were observed for axenic compared to nonaxenic N. *pungens* culture. Antisera against f. *multiseries* did not cross react with f. *pungens*, and antisera against f. *pungens* do not cross react with f. *multiseries*, even at a low dilution of 1:10. There were no cross reactions of the f. *pungens* antiserum with the 6 other phytoplankton species tested (*Nitzschia pseudodedlicatissima, Cylindrotheca closterium, Bacillaria spp., Chaetoceros spp., Gyrosigma spp.,* and an unidentified chlorophyte). Additional species will be tested for cross reactivity. Live cells and those frozen at -80°C, or preserved in 2% glutaraldehyde/paraformaldehyde, 2% borate-buffered formalin or 2% paraformaldehyde, showed a brighter FITC fluorescence than cells preserved in Lugol's iodine, 0.5% glutaraldehyde, 2% formalin-acetic acid, or frozen at -20°C. Immunofluorescence shows great promise as a technique to distinguish between and to quantify the 2 forms of *N. pungens*.

PHYTOPLANKTON MONITORING IN NOVA SCOTIA

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In October 1991 the Aquaculture Association of Nova Scotia received funding from the Nova Scotia Department of Fisheries to continue a phytoplankton monitoring program initiated by the Inspection Services Branch of the Department of Fisheries and Oceans (DFO). Phytoplankton monitoring workshops were conducted for the shellfish growers on the South and Eastern Shores in November/December 1991 and for those in the Cape Breton area in March 1992. Unlike other phytoplankton monitoring programs in the Maritime Provinces, the growers in Nova Scotia are responsible for taking the water samples and arranging for their delivery to the Scientific and Technical Services Laboratory at DFO in Halifax. If toxic algal species are observed, the growers are also requested to send in shellstock samples for toxin assessment. The cooperation of DFO inspectors has greatly facilitated the transport of samples form the more distant areas. As of March 1, 1992, 18 growers were enroled in the program and 80 samples from 15 sites have been examined. The most common potentially toxic species was *Dinophysis norvegica* which occurred at 11 of the 15 sites. The highest concentrations of *Dinophysis spp.* were observed at Indian Point in Mahone Bay (32,000 cells/l) in October 1991; toxicity tests, however, indicated no detectable diarrhetic shellfish poison (DSP) in the mussels. In general, *Dinophysis spp.* numbers decreased through the fall, but low concentrations (< 1000 cells/l) were observed at several sites during the winter months. The program will continue through the spring and summer to keep growers informed of the presence of toxic phytoplankton, and to determine whether monitoring can give adequate warning of impending shellfish toxicity.

BIOCHEMICAL AND MOLECULAR APPROACHES TO THE STUDY OF GENETIC VARIATION IN THE TOXIGENIC MARINE DINOFLAGELLATE GENUS *ALEXANDRIUM*

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To determine stable levels of genetic variation which might be useful in the characterization of populations of the toxigenic marine dinoflagellate *Alexandrium*, isolates from diverse geographical locations were subjected to nucleotide sequencing and analysis of their respective toxin composition. The nuclear-encoded small-subunit ("18S") ribosomal RNA gene (ssu rDNA) of a "classic" (but non-toxic) reference isolate of *A. tamarense* (NEPCC 183 = Ply 173) from the Tamar estuary, England, the species type locality, was sequenced and compared with four *A. excavatum* isolates form the St. Lawrence estuary in eastern Canada. Nuclear genes encoding the ssu rRNA were amplified by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers and cloned into the plasmid vector pUC18. The nuclear gene for the 18S rRNA of *Alexandrium spp.* consists of 1800 nucleotides inclusive of primer regions. The 18S rDNAs of *Alexandrium* clones exhibit an unusual level of variability at nucleotide position 576 (within the "530 loop" in *Escherichia coli* 16S rDNA numbering), expressed as a deletion in isolate NEPCC 183. Sequence alignment with 18S rDNA from the dinoflagellate *Prorocentrum micans* requires at least twenty insertion/deletions, and maximum identity is 87.0%. Alignement of the *A. tamarense* sequence with other *Alexandrium* 18S rDNAs indicates that the non-toxic isolate is rather divergent form the highly toxic clones of *A. excavatum* from the St. Lawrence estuary, despite its morphological similarity. High-performance liquid chromatography of toxin components of cultured isolates and natural phytoplankton assemblages of

Alexandrium spp. generally indicated similarity between wild populations and unialgal cultures of isolates from the same location. Clustering algorithms and principal factor analysis were used to group *Alexandrium* populations according to their respective toxin profiles. Isolates of *A. excavatum* from the St. Lawrence estuary were particularly rich in N-sulfo-carbamoyl toxins C_1/C_2 (>50 mole %), but also contained high relative levels of neosaxitoxin and saxitoxin, in addition to low amounts of gonyautoxins GTX_{1-4} (<5 mole % of total toxin). The frequent lack of congruence of biochemical and molecular markers with conventional morphological criteria used to separate the more than twenty described *Alexandrium* species, and the tendency for phenoplastic modifications in culture, indicate that considerable intragenetic heterogeneity is not expressed phenotypically. The evidence supports a complex species model for *Alexandrium*, with breeding groups of unknown reproductive affinities, rather than a rigid morphometric interpretation.

ÉTAT DES RECHERCHES SUR LA TOXICITÉ DES MOULES ET DES HOMARDS DANS LA BAIE DE GASPÉ

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Depuis 1987, nous avons entrepris des expérimentations visant à décrire la dynamique spatio-temporelle de la toxicité PSP dans les moules (*Mytilus edulis*) d'élevage et de gisement naturel, en conjonction avec les efflorescences d'*Alexandrium excavatum*. Des moules ont été placées en suspension dans les eaux de la baie de Gaspé à différentes profondeurs; des échantillonnages réguliers ont été pratiqués en vue des dosages de toxines par bioessais sur souris. Les résultats ont montré que la dynamique d'intoxication-détoxication des moules variait selon l'origine des moules, la profondeur, l'éloignement de la berge, et selon qu'elles aient ou non été déplacées du fond vers la surface. Ces observations ont permis d'expérimenter une adaptation de la technique traditionnelle de culture en suspension permettant d'atténuer l'accumulation des toxines. En 1991, nous avons également pratiqué quelques expérimentations sur la toxicité des homards (*Homarus americanus*) provenant d'une population naturelle de la baie de Gaspé, dont l'hépatopancréas contenait des quantités appréciables de toxines. Des homards ont été gardés en stabulation prolongée pendant des périodes variant de 15 jours à 5 mois. Les homards provenaient tous de la même zone de pêche. Il est apparu qu'une stabulation de 50 jours ne permettait pas de détoxiquer efficacement les homards (plus de 100 µg STXeq/100g). Même après 5 mois de stabulation, environ 90% des homards contenaient des quantités détectables de toxines. La variabilité à l'intérieur des groupes d'apparence homogène s'est révélée très grande, ce qui complique les échantillonnages.

MESODINIUM RUBRUM: PROBABLE CAUSE OF RED COLORATION IN CULTIVATED MUSSELS

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In the early spring of each of the past two years cultivated mussels in some of the bays of Nova Scotia's Eastern Shore have developed a strong reddish color in the digestive tract and associated liquid. Although there was no apparent toxicity associated with the red coloration, there was concern among the mussel growers. Examination of red colored mussels using epifluorescence microscopy revealed the presence of small (<10 µm) particles with fluorescence characteristics identical to those of phycoerythrin. This fluorescence was not observed in extracts from "control" mussels grown in filtered seawater. The excitation and emission fluorescence spectra of extracts from the red mussels were analyzed using a spectrofluorometer. This revealed a unimodal fluorescence signature characteristic of phycoerythrin that was not present in the control mussels. Freshly collected water samples from the affected areas were examined and showed the presence of large numbers of the "red water" ciliate *Mesodinium rubrum* that exhibited the phycoerythrin fluorescence under epifluorescent illumination. *Mesodinium rubrum* in a small (ca. 50 µm) protozoan that harbors an obligate algal cryptomonad symbiont, which contains phycoerythrin as an accessory photosynthetic pigment. Phycoerythrin is a water-soluble pigment that appears to be relatively refractory in the digestive tract of the mussel. It appears that grazing of blooms of the non-toxic, ciliate *Mesodinium rubrum* results in transfer of phycoerythrin pigment to the mussels. One reason for the initial difficulty in observing the ciliate was probably the well documented fragility of *Mesodinium rubrum*, both in freshly collected samples and in conventional fixatives. It is notable that *Mesodinium rubrum* blooms have been recorded over a wide variety of marine environments since

early observations by Charles Darwin in the late 1800's. However, there are remarkably few reports of the effects on predators of grazing these ubiquitous marine ciliates. Research is in progress to document the effects of grazing and depuration of cultivated mussels in water containing *Mesodinium rubrum*.

TEMPORAL AND SPATIAL VARIATION IN PSP IN SOUTHWESTERN N.B. 1943 TO THE PRESENT

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The Department of Fisheries and Oceans Inspection Branch at Blacks Harbour, N.B. has been monitoring Paralytic Shellfish Poisoning in clam and mussel meats in Charlotte and Saint John Counties for 49 years. This data is now in a database, and is being used to help predict PSP blooms and to help maintain an efficient and meaningful sampling program. Twenty-three Biotoxin Management Areas have been established, of which 19 are monitored regularly for PSP. Approximately 900 samples are presently taken each year. PSP values from soft shelled clams (*Mya arenaria*) for each management area have been averaged over two week periods for the years they were sampled. A maximum value for each two week time period and data for 1991 have also been graphed. A wide range of PSP severity and frequency of blooms is seen between areas, often in close proximity. Although the Bay of Fundy is closed for the harvesting of blue mussels (*Mytilus edulis*), samples are taken for PSP bioassay in order to help predict the blooms which close the clam flats. Data from these samples help confirm blooms seen in the clam samples. These samples also help screen for domoic acid.

THE MARINE KILLERS: DINOFLAGELLATES IN ESTUARINE AND COASTAL WATERS

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Records of massive fish kills and paralytic shellfish poisoning (PSP) in Europe and North America go back to the 17th century. But, it was not until the 1940s when the relationship between PSP, red tide and toxic dinoflagellate *Gonyaulax* was established. Recent records show that PSP and related poisons caused by toxic dinoflagellates in coastal waters and estuaries, are a world-wide problem. Diarrhic shellfish poisoning (DSP) and neurotoxic poisoning (NSP), believed earlier as bacterial or viral infections are now shown to be caused by the other toxic dinoflagellates such as *Dynophysis*. The shellfish most often involved in the poisoning are mussels and clams. Other dinoflagellates, *Gyrodinium*, occasionally cause massive fish kills in vast coastal areas, resulting in fishery and economic losses. Factors promoting toxic dinoflagellate bloom development and PSP/DSP outbreaks are not fully understood. In previous studies, temperature was considered as the principal factor influencing dinoflagellate blooming. Recent studies showed that other factors such as salinity, sunlight, freshwater runoff and water stability are also important. Pollution from land drainage and sewage discharge in inshore waters were also implicated. Current knowledge indicates that although chemical and biotic factors are important for *in-situ* growth of dinoflagellate cells, convergence by thermal and tidal fronts is essential for cell accumulation and bloom development. Advances in physical oceanographic research, modelling and remote sensing enabled the detection of fronts and bordering eddies with high precision. There is a potential for an increased use of these technological advances in predicting and monitoring the bloom development. The present poster overviews the history and distribution of toxic dinoflagellates, and the physical factors influencing bloom development and PSP/DSP outbreaks. Future research needs to improve the predictability and control of this world-wide hazard are also discussed.

NATIONAL INVENTORY OF PHYTOPLANKTON TAXONOMIC AND ECOLOGICAL STUDIES

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The Phycotoxins Working Group of the Department of Fisheries and Oceans has initiated a national inventory of phytoplankton taxonomic and ecological studies. The objective of the inventory is to provide for exchange of information on phytoplankton distribution and abundance data, and associated physical and chemical measurements. Information collected on studies includes purpose, dates, locations, types of analyses conducted, and data format and availability. Reports and papers generated from the studies, along with names of contact people are also included. A test version of the inventory has been implemented in a DBASE IV database. Procedures are also being developed for data transfer by electronic mail (currently VAXMail on DFONet). Given sufficient interest, we will canvas all institutions that may have relevant data. The full database will be available for exchange by a variety of media, including DOS disks as DBASE or ASCII files, and electronic mail. Periodic updates would be conducted.

IMPACT OF TOXIC DINOFLAGELLATE BLOOMS ON THE RECRUITMENT OF FINFISH POPULATIONS

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Recent investigations have established that survival of larval fish is compromised in presence of *Alexandrium excavatum*, a common toxic dinoflagellate in Eastern Canada. We test the hypothesis that toxic dinoflagellate blooms could jeopardize recruitment to coastal finfish populations by reducing young fish survival. To this end, we evaluate the relations between toxicity indices and recruitment of herring populations in the southern Gulf of St. Lawrence and in Nova Scotia. The results do not support the hypothesis, but the possibility that major blooms of toxic dinoflagellates can contribute to decimate a year-class cannot be ruled out yet.

AN OUTLINE OF EVENTS AND DFO INSPECTION ACTIVITIES RELATING TO DSP INTOXICATION OF CULTURED MUSSELS IN SCOTIA-FUNDY DURING 1991

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Early in the spring of 1991, Inspection Branch, DFO encountered suspected shellfish poisoning of several people. The shellfish involved came from Ship Harbour, N.S. and were harvested around April 22. Concentrated DSP extracts of the shellfish killed mice on intraperitoneal injection and gave a positive test with the UBE DSP-check kit immuno-test. The chemical analysis (Fenwick Laboratories) indicated that the extracts did not contain okadaic acid (OA) or DTX-1. The shellfish at this time were found to contain a red pigment, the source of which could not be identified, and were reputed by some to have a peppery taste. Neither characteristic appeared to be related to the observed toxicity. DSP-type extracts frequently gave a peculiar CNS effect when injected into mice, but less frequently lethal. Extensive evaluation of shellfish stocks indicated that the "unidentified toxic factor" (UTF) was widespread, but was of no known toxicologic significance. In June, partly due to the intensified monitoring, DSP activity was detected in mussels through the combined mouse test and UBE DSP-check kit. The presence of significant amounts of OA and DTX-1 were found in Ship Harbour mussels and substantial DTX-1 in Mahone Bay mussels (Fenwick Laboratories). The Ship Harbour and the Mahone Bay DSP peaked about mid-July and declined slowly to insignificance at about the end of August. No source organisms could be established in either case. Phytoplankton monitoring indicated low numbers of Dinophysis.

UPTAKE AND EXCRETION OF PARALYTIC SHELLFISH TOXINS BY LOBSTER FED SCALLOP DIGESTIVE GLANDS

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Lobster, *Homarus americanus*, sampled from the Bay of Fundy in 1990 were reported to have levels of paralytic shellfish poisoning (PSP) toxins greater than 80 µg saxitoxin (STX) equivalent/100 g wet wt in hepatopancreas (W. Watson-Wright *et al.* 1991, Can. Tech. Dept. Fish. Aquat. Sci. No. 1799, 27). The present study is concerned with the uptake and excretion of PSP toxins by lobsters fed food contaminated with PSP toxins. Lobsters (250-300 g) were held in individual compartments with flowing sea water at ambient temperature (12-14°C). Digestive glands of scallop, *Placopecten magellanicus*, containing PSP toxins (4000 µg STX equiv/100 g wet wt) were fed to the lobsters two times per week for 16 weeks. Six lobsters were sampled periodically. The PSP toxin concentrations of stomach, hepatopancreas and tail muscle were determined by mouse bioassay and the mouse neuroblastoma cell culture technique (see poster by J.F. Jellet *et al.*). PSP toxins were not detected in the tail muscle. PSP toxins were found in the stomach and hepatopancreas after two weeks of feeding. There was a large variation in the levels of PSP toxins in the hepatopancreas between individuals sampled from the same time period. The mean concentration of PSP toxins in the hepatopancreas were not significantly different in lobsters sampled from week 2 through to week 16 (>500 µg STX equiv/100 g wet wt, range 275-3200 µg STX equiv/100 g wet wt). Another group of lobsters were fed scallop digestive glands 3 times a week for 3 weeks, then fed food not contaminated with PSP toxins. Lobsters were sampled periodically to determine the excretion rate of the PSP toxins. The results suggest that wild lobsters can accumulate PSP toxins through their food, but the toxins are readily excreted.

RÉSULTATS DE TROIS ANNÉES DE MONITORAGE DU PHYTOPLANCTON TOXIQUE DANS L'ESTUAIRE ET LE GOLFE DU SAINT-LAURENT

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Depuis 1989, une dizaine de stations situées dans l'estuaire et le nord du Golfe St-Laurent sont échantillonnées entre juin et novembre afin de déterminer la présence des microalgues toxiques. Les résultats des trois années d'échantillonnage indiquent que les floraisons d'*Alexandrium* spp. et de *Dinophysis* spp. surviennent à la fin juin et varient annuellement en intensité. *Alexandrium* spp. est principalement confinée au panache des rivières Manicouagna et Aux-Outardes ainsi qu'au courant de Gaspé dans la région étudiée. De faibles concentrations d'*Alexandrium* spp. ont été enregistrées sur la Basse Côte-Nord ainsi qu'aux Iles-de-la-Madeleine. *Dinophysis* spp. est peu abondant mais présent sur l'ensemble du territoire. *Nitzchia pungens* forma *multiseries* n'a pas été observée dans toute la région d'étude.

THE SCOTIA-FUNDY PHYTOPLANKTON MONITORING PROGRAM; THE NOVA SOCTIAN COMPONENT

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The Nova Scotian component of the Scotia/Fundy Region phytoplankton monitoring program began in October of 1988 and was completed in December 1991. Five coastal sites were sampled on a regular basis; 4 along the Atlantic coast and 1 in the Bay of Fundy. Over the 3 year period, samples were collected from each site on approximately 30 different dates each year. A long-term temperature recorder was located at each site and on each sampling date a depth profile of salinity, temperature and *in vivo* fluorescence was recorded. Samples were collected at each of 3 depths for determination of salinity, extracted chlorophyll, suspended particulate matter, and plant nutrients. A subsample was removed for phytoplankton identification and enumeration. A vertical net tow, 20 µm mesh, provided an integrated sampling of the

phytoplankton species present. Database management is handled on a personal computer with a 80286 CPU using Version 2.0 of FoxPro. The system will be demonstrated on a 80386 based portable. Selected data from 1990 for the stations in Ship Harbour and Digby are displaced.

ÉVALUATION DE LA SPÉCIFICITÉ DES ANTICORPS ANTI-ACIDE DOMOÏQUE

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L'acide domoïque (AD) est la toxine marine qui a été à l'origine des 150 cas d'intoxications et des quatre décès survenus au Québec à l'automne 1987 suite à la consommation des moules contaminées. Dans le but de développer un outil de dépistage et/ou de dosage de l'AD dans les échantillons de mollusques, nous avons couplé l'AD à différentes protéines porteuses pour servir d'immunogène dans la production d'anticorps (Ac.) chez le lapin. La cinétique de production des Ac. a été établie en mesurant les titres sériques au cours des immunisations, à l'aide d'une techniques d'ELISA modifiée utilisant des bâtonnets de polystyrène comme support de l'antigène. Les lapins immunisés avec le complexe poly-D-lysine, l'espaceur FNPS et l'AD, ont été les seuls à produire des taux d'Ac. suffisants au cours des immunisations. En conditions standardisées, le titre du pool d'échantillons de sérums positifs était de 1/800, comparativement aux contrôles. Les Ac. produits étaient spécifiques à l'AD puisqu'ils ne réagissaient pas avec toutes les autres molécules utilisées comme contrôles négatifs. Il a été difficile de produire des immunoglobulines anti-AD puisque seulement 20% des lapins ont produit des Ac. et un seul avait des titres convenables. Malheureusement ces Ac. anti-AD ne s'absorbent pas avec l'AD en solution, mais seulement avec l'AD fixés sur support solide, suggérant que ces Ac. ont une faible affinité. En somme, il semble possible, mais difficile de produire des Ac. anti-AD de bonne qualité en immunisant des lapins avec l'AD couplé aux molécules porteuses.

PHYTOPLANKTON MONITORING IN THE SOUTHWEST BAY OF FUNDY

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During 1987, a study of twelve sites was initiated to observe phytoplankton populations in the southwest Bay of Fundy following the introduction of a salmonid aquaculture industry that was rapidly expanding. The study was begun with three purposes: to establish baseline environmental data, to act as an early warning for harmful algal occurrences and to help manage industries. This programme was expanded to seventeen sites in 1988 following the domoic acid outbreak in eastern Prince Edward Island and the subsequent establishment of phytoplankton monitoring programmes on Canada's Atlantic and Pacific coasts. Although more than 150 different species of organisms have been observed, data is presented from concentrations of*Alexandrium fundyense, Nitzschia pseudodelicatissima*and *Dinophysis spp.*, organisms implicated in PSP, ASP, and DSP outbreaks. Results are presented from a typical inshore sampling site (Lime Kiln Bay, southwest Bay of Fundy). Highest concentrations (since sampling began) of *A. fundyense, N. pseudodelicatissima* and *Dinophysis spp.*, in Lime Kiln Bay were observed in 1990 with 58,000, 112,000 and 2,240 cells/liter, respectively. These organisms occur annually in the Bay of Fundy with highest concentrations of *A. fundyense* generally from June to August, *Dinophysis* from July to October and *N. pseudodelicatissima* from August to October.

LABORATOIRE PHYCOTOXINES ET NUISANCES DE NANTES (IFREMER)

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Le laboratoire phycotoxines et nuisances a pour axe majeur de recherche les processus de contamination des bivalves par les phycotoxines. De ce thème principal peuvent se dégager trois orientations d'études, qui sont: (1) les causes de la variation du contenu toxinique dans les algues et les coquillages contaminés, (2) les origines de la production toxinique et (3) les mécanismes de bioaccumulation/élimination des toxines chez les mollusques. Pour ce faire, le laboratoire a développé des compétences en ce

qui concerne les études *in vivo* et *in vitro* du plancton toxique, l'analyse des toxines et l'écophysiologie des coquillages.

OCCURRENCES OF PHYCOTOXINS AND RELATED PHYTOPLANKTON IN THE COLD IN THE SOUTHEASTERN GULF OF ST. LAWRENCE, CANADA

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Observations of domoic acid (DA) in shellfish and plankton and of incidences of *Nitzschia pungens* f. *multiseries* and other toxic algae in the southeastern Gulf of St. Lawrence indicate, unexpectedly, that such events can take place in very cold, ice-covered water. In the cold, shellfish can become slightly toxified by DA, probably from *N. pungens* f. *multiseries*, but perhaps other sources as well. *N. pungens* f. *multiseries* in culture can grow, produce and retain DA when shifted down from growth temperature of 13 to 5 or 0 and from 5 to 0°C. DA was also found in plankton from a sub-ice spring bloom (water temperature -1.5 to 0°C); these samples did not contain *N. pungens* f. *multiseries* or any other suspected DA producer; cell counts of a *Fragilaria sp.* were best correlated with changes in DA concentration. Substantial sub-ice populations of *Alexandrium excavatum* and an ubiquitous photosynthetic euglenoid are also reported. The euglenoid is an obligate psychrophile and is related to toxic forms. The DA levels observed do not threaten acute intoxication of humans, but the effects of chronic low level DA exposure and the possibility of other DA producers require study.

PHYSIOLOGICAL ECOLOGY OF *DINOPHYSIS NORVEGICA*, A REDWATER BLOOM SPECIES, IN BEDFORD BASIN, NOVA SCOTIA

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The relationships between photosynthesis and photosynthetic photon flux densities (PPFD, P-I) in *Dinophysis norvegica*, a dinoflagellate, were studied during a redwater bloom during July - August 1990 in Bedford Basin, a coastal embayment off Nova Scotia. Dinoflagellates such as *Gonyaulax digitale, Ceratium tripos, Peridinium oceanicum, Prorocentrum minimum* and *Gyrodinium* sp. along with *D. norvegica* contributed to ~50% of the phytoplankton abundance that attained a maximum of 16.7 µg Chl a l^{-1} and 11.93 x 10^6 cells l^{-1}. Photosynthetic characteristics on isolated single species of *D. norvegica* and fractionated natural phytoplankton assemblages with >90% *D. norvegica* were similar: α - the initial slope of P-I curves ranged between 0.013 and 0.047 µg C [µg Chl a]$^{-1}$ h^{-1} [µmol m^{-2} s^{-1}]$^{-1}$, the maximum photosynthetic rate P_m^B between 0.66 and 1.85 µg C [µg Chl a]$^{-1}$ h^{-1} and I_k - the photoadaptive index from 14 to 69 µmol m^{-2} s^{-1}. Carbon uptake rates of *D. norvegica* ranged from 11 to 25 pg C cell^{-1} h^{-1} which were lower compared to other dinoflagellates.

HARMFUL MARINE PHYTOPLANKTON OF CANADIAN ATLANTIC WATERS

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Data from about 70 publications on marine phytoplankton in the Northwest Atlantic waters were reviewed. A broad overview of the ecology of twelve phytoplankters is presented, including *Alexandrium tamarense* and *A. fundyense* that cause PSP episodes; *Dinophysis norvegica, D. acuminata, D. acuta, Prorocentrum minimum* and *P. micans* implicated in DSP episodes and *Nitzschia pungens* f. *multiseries, N. pseudodelicatissima* -the neurotoxin, domoic acid, producing diatoms causing amnesic shellfish poisoning. A comparison with other regions showed that a few of these algae at times occur in toxic bloom proportions in our waters. Also, a potential for the development of blooms of other species exists. Monitoring programmes coupled with studies on the physiological ecology of these potentially harmful algae would be instructive and beneficial to mariculture industry.

EFFECTS OF IRRADIANCE ON THE GROWTH AND TOXICITY OF *ALEXANDRIUM TAMARENSE* IN CAGE-CULTURE TURBIDOSTATS

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The effects of irradiance on the growth rate and toxicity of the dinoflagellate *Alexandrium tamarense* were studied using a cage-type turbidostat culture system. This method allowed cell division rate to be monitored continuously while maintaining a stable culture density and a nutrient replete environment. The culture were subjected to step-function changes in irradiance, both decreases and increases. Samples were withdrawn daily and analyzed for cellular toxin content using a Sullivan-train HPLC system (courtesy of the NRC Institute for Marine Biosciences, Halifax). Toxin production rates were also calculated. The relationship of cellular toxin content and production rate to cell division rate and irradiance is discussed.

AN IDIOTYPIC-ANTI-IDIOTYPIC COMPETITIVE IMMUNOASSAY FOR QUANTISATION OF OKADAIC ACID

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A competitive indirect enzyme-linked immunosorbent assay for the measurement of okadaic acid, a marine toxin, was developed. The assay uses a murine monoclonal anti-idiotypic antibody bering an internal image of okadaic acid epitope to capture an anti-okadaic acid monoclonal antibody in the presence of free okadaic acid. Bound

anti-okadaic acid antibody is detected with peroxidase-conjugated anti-mouse immunoglobulin antiserum. If present, free toxin will lessen the amount of anti-okadaic acid antibody binding to its corresponding anti-idiotypic antibody in a dose dependent manner that can be quantified from the standard curve. The assay permits reliable measurement of okadaic acid in the 9-81 ng/ml range. The intra- and interassay coefficients of variation in the measurement of OA in the toxin spiked mussel samples average 9% and 12%, respectively. The assay is rapid, accurate, reproducible and relatively simple to perform. It may be of potential use to laboratories involved in monitoring the toxin levels in plankton, seafood or sponges.

PRODUCTION D'ACIDE OKADAÏQUE ET DE DINOPHYSITOXINE 1 PAR *PROROCENTRUM LIMA* ERHENBERG: INFLUENCE DU MILIEU DE CULTURE ET DU STADE DE CROISSANCE

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Trois souches de *Prorocentrum lima* productrices de toxines diarrhéiques et originaires de la baie de Vigo en Espagne ont été cultivées dans trois milieux de croissance contenant différentes quantités de matières organiques. Le milieu F/2 est utilisé comme référence par rapport à un milieu F/2 dépourvu d'EDTA remplacé par une dose de 30 mg/l d'acides humiques commerciaux et un milieu Erdschreiber à base d'extrait de compost. La présence de matières organiques dans le milieu montre une influence bénéfique nette sur le taux de croissance et le nombre total de cellules en phase stationnaire. Des échantillons d'algues ont été prélevés dans ces trois cultures à différents stades de croissance afin d'en évaluer le contenu en toxines par analyse HPLC selon une méthode adaptée d'après Yasumoto par Braekman et Pereira. Les contenus en toxines sont discutés en référence aux courbes de croissance des algues et à l'effet bénéfique des matières organiques sur les totaux cellulaires en vue d'optimaliser la production de toxines diarréhéiques par *P. lima.*

NEW OCCURRENCES OF PARALYTIC SHELLFISH POISONING TOXINS IN THE SOUTHERN GULF OF ST. LAWRENCE, CANADA

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Although prevalent in other parts of the Gulf of St. Lawrence, paralytic shellfish poisoning (PSP) had not been documented in the southern reaches of the Gulf until 1988, when levels >1000 µg STXeq·100 g^{-1} were measured in bar clams from the Miramichi estuary (New Brunswick). Prior to the domoic acid crisis of 1987, PSP monitoring in the southern Gulf was minimal and earlier occurrences of low levels of PSP toxins may thus have gone unnoticed. Since the summer of 1988, PSP has reoccurred, but the geographic pattern and the species affected have been different. From 1989 on, northern moonshells, a carnivorous gastropod, exhibited the most consistent pattern of toxification, with little seasonal variation. Interannual variations in toxicity appear to be related to their molluscan shellfish prey. Available phytoplankton data do not provide clear insight into the cause of the PSP problem. In the southern Gulf, ***Alexandrium excavatum***, the species historically associated with PSP in the Gulf of St. Lawrence, appears to be most abundant under the ice during the winter, a period when sampling for molluscs is severely hampered. It is possible that, during the 1988 PSP episode, moonshells accumulated large amounts of PSP toxins by feeding on toxified bivalves and that they are slowly depurating this toxin burden over several years. Alternatively, moonshells may bioconcentrate PSP toxins by preying on bivalves contaminated at levels below the mouse bioassay detection limit (ca. 40 µg STXeq·100 g^{-1}).

WORKING GROUP REPORTS

MANDATE OF THE WORKING GROUPS

Chairman: Maurice Levasseur

A. ORIGINE AND PROPAGATION OF HARMFUL ALGAE

GROUP 1: The American West Coast domoic acid crisis.

- Implications for the Canadian Waters
- Comparison with the Canadian East Coast problem
- What should be done (Research recommendations, monitoring...)?
- Need of a standard method for culturing?

GROUP 2: The Canadian East Coast DSP problem.

- Estimation of the extent of the problem
- Are *Dinophysis* spp. responsable for DSP production?
- Culturing problems
- Research recommendations

B. TRANSFER OF PHYCOTOXINS IN THE FOOD WEB

GROUP 3: Transfer of phycotoxins in the benthic food web.

- Is there any efficient depuration techniques with good potential for commercial application?
- Toxin distribution in the organisms. Do we know enough about toxins distribution in different tissus of commercial organisms (e.g. lobster, scallop)?
- Considering these new problems, is the Canadian monitoring and surveillance program adequate?
- Does algal toxicity have a significant effect (e.g. growth, reproduction...) on shellfish species of commercial values?

GROUP 4: Transfer of phycotoxins in the pelagic food web.

- What is the effect of algal toxicity on pelagic grazers (zooplankton, fish larvae)?
- Does algal toxicity have an effect on recruitment of commercial species?
- Is algal toxicity transferred to adult fish species and what is its effect it has on adult fish species?

C. DETECTION AND QUANTIFICATION OF TOXIC ALGAE AND PHYCOTOXINS

GROUP 5: Detection and quantification of toxic algae and toxins

- PSP toxins standards production: Assessment of the situation
- Other toxin standard: needs
- Needs for inter-laboratory calibration
- Development of new methods for detection of toxins
- New and rapid methods of identification of harmful algae

D. MONITORING AND PREDICTION OF HARMFUL EVENTS

GROUP 6: Monitoring toward year 2000

- Are monitoring program useful?
- What is needed to predict blooms of harmful species?
- Need for models?
- The water ballast concern: Is deballasting an underestimated problem in Canadian Waters?

Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #1

THE AMERICAN WEST COAST DOMOIC ACID CRISIS

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INTRODUCTION

The occurrence of domoic acid along the west coast of the United States in 1991 confirms the risk of its occurrence along the Pacific coast of Canada. It is apparent that domoic acid is a much more significant and widespread problem than the relatively restricted and manageable occurrences in Prince Edward Island would suggest.

The event differed in fundamental ways from previous episodes in Atlantic Canada: a) the organisms responsible for producing the domoic acid (i.e. *Nitzschia pungens* f. *multiseries* on P.E.I. vs. *Pseudonitzschia australis* and other unknown sources on the west coast); b) domoic acid was found in blue mussels plus other minor molluscan shellfish on P.E.I., and in anchovies, brown pelicans, Brandt's cormorants, Dungeness crabs and razor clams on the west coast; c) blue mussels on P.E.I. rapidly depurate their accumulated domoic acid because it is found predominantly in the viscera, whereas in the case of razor clams the domoic acid is distributed throughout the body, perhaps accounting for its apparent persistence in the animal; d) domoic acid appears predominantly during August to November on P.E.I., and although the problem was first detected in September on the west coast, there is evidence that domoic acid may also have been present earlier in the year; and e) the problem is confined to relatively sheltered embayments on P.E.I. compared to more exposed areas on the west coast. In the end, the only major similarities between the two situations are the presence of domoic acid and the potentially devastating impact on the fishing industry and on human health. The differences between the east and west coast situations emphasize the need for research to establish the nature of these differences, including research in Atlantic Canada to address potentially overlooked sources of domoic acid there. It is also recognized that a problem with the U.S. fishery will have spill-over effects on the Canadian fishery in relation to potential loss of markets and loss of consumer confidence in the product.

Given the now geographically widespread and international nature of the domoic acid problem, the working group recognized that closely linked efforts are needed in Canada and the United States to carry out the required research to determine the sources of the domoic acid, to understand the physical and biological mechanisms that lead to domoic acid accumulation in marine animals, to standardize detection methods, and to establish optimum monitoring programs.

Domoic acid along the Pacific coast of the U.S. was first manifested in mortality of pelicans and cormorants in Monterey Bay, California, in September 1991. The source of toxicity was anchovies which had been feeding on the marine diatom *Pseudonitzschia australis*. Subsequent analysis of anchovies demonstrated that domoic acid was present not only in the gut (typically to 200 ppm; maximum: 2300 ppm), but had been incorporated into the flesh of the fish. Culture experiments of *P. australis* confirmed that it produces domoic acid. This was the first documented case of marine food web effects by this toxin.

Shortly after the razor clam season opened in Oregon and Washington in late October, domoic acid was found in clams from Washington (28-47 ppm), leading to closures for shellfish harvesting. Subsequently, traces of domoic acid were found in Dungeness crab from Washington and Oregon and in razor clams from Alaska (< 6 ppm). Analyses showed that domoic acid was distributed throughout the body of razor clams, with highest values occurring in the foot and mantle. In contrast, domoic acid appears to be restricted to the viscera of crab, except on cooking in boiling water, when it may be transferred to the meat. The source of domoic acid in Washington, Oregon and Alaska has not been established. Analysis of canned samples from April, May, June and October 1991 showed that clams along the Washington coast were contaminated in all those months. Analysis of home canned samples has also shown that domoic acid was present as early as 1985. Peak levels of domoic acid found in 1991 were in excess of 154 ppm in razor clams and up to 40 ppm in Dungeness crab and 100 ppm in Stone crab (Morro Bay, CA).

An unknown number of people suffered from Amnesic Shellfish Poisoning (ASP) as a result of this event, but at least two presented significant neurological symptoms.

The working group discussed topics suggested by the organizing committee. Specific questions posed were:

- What are the implications for Canadian waters?
- How does the west coast problem compare to Canadian east coast experience?
- What should be done (research, monitoring ...)?
- Is there need for a standard method for culturing?

The working group recommendations follow from these questions and other issues that were identified.

RECOMMENDATIONS

1. Determine the source(s) and location(s) of domoic acid on the west coast of North America.

The source of domoic acid in Monterey Bay, California, has been reasonably well documented as the diatom *Pseudonitzschia australis*. However, there is no evidence regarding the source and location of domoic acid found in other areas (California, Oregon, Washington and Alaska). There may have been a single source or multiple sources. Domoic acid remains at high levels (20 - 50 ppm) in razor clams in Washington and Oregon in the absence of known domoic acid producers. The possibility should therefore be investigated that there are micro-organisms which produce low levels of domoic acid throughout the year, thereby chronically exposing shellfish to the toxin.

2. Expand efforts to identify other potential sources and locations of domoic acid on the east coast of North America.

The nature of the domoic acid incident on the west coast, including the widespread occurrence of the toxin in a variety of marine organisms, reveals the potential for the production of domoic acid by unrecognized sources in eastern Canada. Evidence of this possibility already exists in, for example, the occurrence of domoic acid under sea ice in the absence of *Nitzschia pungens*, and the toxicity of scallops found in relatively deep water on Georges Bank.

3. Intensify domoic acid monitoring of commercially important shellfish, crustaceans and planktivorous finfish along the west coast of Canada.

The United States data show that domoic acid can be found in many unsuspected species. The evidence also indicates that mussels, the primary monitoring organisms in the DFO phycotoxin inspection program, rapidly depurate domoic acid. While it is recognized that Inspection Branch has conducted some tests of additional species, such as Dungeness crabs and razor clams, more widespread geographic coverage of these and other economically important species is required to establish the presence or absence of domoic acid along the British Columbia coast.

There is evidence that the acid extraction procedure leads to loss of toxin in the sample, particularly if there is a delay in sample analysis. Extraction and analytical methods should be standardized, in particular, the use of the methanol:water extraction method, and the regional inspection laboratory should be fully equipped to perform these analyses using a standard protocol.

4. Investigate pathways of domoic acid transfer through the food web.

The west coast incident has revealed varied and complex transfer pathways of domoic acid through a variety of species, such as:

phytoplankton → finfish → avians

unknown source → crabs → unknown consumers (possibly including humans)

unknown source → razor clams → humans and other unknown consumers.

This indicates the potential for other unsuspected pathways to humans and other marine organisms, with currently unquantifiable potential for harm.

5. Determine the ecotoxicological impact of domoic acid on selected marine species.

Domoic acid is a potent neurotoxin and some marine species may be vulnerable to it. Its widespread distribution among marine species on the west coast suggests that domoic acid may disrupt established population dynamics, including recruitment of commercially important fish species. The potential for domoic acid contamination of marine products used for the production of fish and chicken feed should also be assessed.

6. Examine the effects of continued low level exposure to domoic acid.

The prevalence and persistence of low levels of domoic acid in many commercially and recreationally harvested marine species, presently documented back to 1985, underscores the importance of understanding the potential effects of chronic exposure on both invertebrate species and humans. This requires a series of long-term laboratory studies and may also lead to a reassessment of the current tolerance level for domoic acid in seafood. It is possible that this could result in a requirement for different tolerance levels for different harvested species.

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Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #2

DIARRHETIC SHELLFISH POISONING IN CANADA

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INTRODUCTION

Diarrhetic shellfish poisoning (DSP) in humans is a severe (although not fatal) gastrointestinal intoxication syndrome resulting from the consumption of contaminated shellfish. Outbreaks of DSP have been common on a global scale and hundreds of cases have been reported, particularly from Japan and northern Europe. Extensive inspection of shellfish products for DSP toxins, as well as the monitoring of toxic phytoplankton blooms, have been instrumental in reducing the number of DSP intoxications in recent years. In North America, no cases of DSP had been confirmed prior to 1990, although there have been several poorly documented suspicious incidents which fit the basic DSP symptomology recorded over the past two decades.

To propose effective research and regulatory strategies for the actual and potential risk of DSP episodes in Canada, this Working Group addressed the following issues:

1) Is the current level of financial and human resources devoted to DSP research commensurate with the public health risk to the consumer and the consequent

negative effect on the economic viability of the shellfish industry?;

2) What is the extent of current knowledge regarding the causes and effects of DSP in Canada?;
3) Are the species responsible for DSP in other countries (e.g., *Dinophysis* spp.) similar or identical to those found in Canadian waters, and are they directly implicated in domestic incidents?;
4) Is the culture of toxic *Dinophysis* spp. for ecophysiological and toxicological studies a worthy goal, and has sufficient effort been expended on this objective?;
5) What are the methodological problems associated with the analysis of DSP toxins and are these difficulties being successfully resolved in Canada?;

The Working Group used the extensive discussions of these fundamental questions as the basis for formulating a series of realistic research objectives.

Present Knowledge and Gaps

1. Distribution and identification of putative DSP toxin-producing species

Toxic phytoplankton species implicated as causative agents of DSP, specifically *Dinophysis* spp. and certain benthic/epiphytic *Prorocentrum* spp., commonly occur in Canadian waters. *Prorocentrum lima* has been occasionally identified in phytoplankton samples from both the Gulf and Scotia-Fundy fisheries regions, while *P. concavum* has been found only in mussel gut contents in low abundance. In the Pacific fisheries region, *D. fortii* and *D. ellipsoides* are widely distributed, although their appearance is not obviously associated with DSP incidents. Along the Atlantic coast, certain *Dinophysis* spp., including *D. acuminata*, *D. acuta*, *D. fortii*, *D. norvegica*, and *D. rotundata*, are often present at low cell densities ($<2{,}000$ cells L^{-1}) throughout much of the ice-free period in nearshore waters. *Dinophysis caudata* made a rare appearance in Nova Scotia in 1990; *D. mitra* and *D.* cf. *ovum* have also been observed in the Gulf region. Occasional summer blooms of *D. norvegica* and *D. acuminata* may attain cell densities $>200{,}000$ cells L^{-1}, and a recent report from Bedford Basin, Halifax gave counts as high as 500,000 cells L^{-1}, but discolouration of surface waters ("red tide") is rarely, if ever, observed. It has not been established whether *Dinophysis* populations are endemic to coastal embayments in Canada, or if they originate off-shore and are transported towards the shore, as is the case for the Atlantic coast of France. There is some circumstantial evidence that *Dinophysis* blooms in the lower estuary and Gulf of St. Lawrence are associated with stratification of the water column, resulting from the formation of a distinct summer pycnocline in surface waters. It was also noted that the major DSP incident in Nova Scotia in 1990 occurred in the aftermath of a major storm, with strong onshore winds and a rapid increase in surface water temperature adjacent to the shore.

As is characteristic for *Dinophysis* spp. from other environments, species prevalent in Canadian waters are subject to a high degree of morphological variability in size, overall shape, the form of the sulcal lists ("wings") and surface ornamentation, with apparent inter-specific gradations. No comprehensive morphological analysis of *Dinophysis* spp. common to eastern Canada is yet available, although cursory analysis suggests that species are morphologically similar to (if not indistinguishable from) their counterparts elsewhere.

Recent work has shown that DSP toxin-producing species can exhibit a variety of morphotypes within and among alternative life history stages. For example, tropical forms of toxic *P. lima* appear capable of cycling between a free-living and an asexual cyst stage, in addition to undergoing sexual reproduction to form zygotic cysts. Among certain *Dinophysis* spp. from European waters, both temporary (asexual) cysts and putative gametes ("small forms") have been reported. The existence of dimorphic *Dinophysis*, with opposing thecal halves of the same cell resembling those of two different morphospecies, has provoked debate regarding the validity of presently described "species". Inconclusive evidence of sexuality in *Dinophysis* cf. *acuminata* from Nova Scotian waters was recently presented, based upon the presence of two trailing flagella in a triflagellate "planozygote". The significance of these observations in a Canadian context remains to be determined.

2. Extent of the DSP problem in Canada

Definitive evidence of DSP cases in Canada is difficult to acquire, as the classic symptoms of DSP (gastrointestinal distress, nausea, vomiting, etc.) can be readily confused with other common causes of seafood poisoning, typically by bacteria or viruses. It is also likely that legitimate DSP cases in Canada go unreported due to public health officials and medical personnel who are unfamiliar with the diagnostic criteria. In addition, the lack of a comprehensive DSP monitoring program and difficulties in the implementation of analytical techniques for DSP toxin analysis have undoubtedly contributed to under-reporting. For example, the cause of a major seafood toxicity incident in the 1970s, which involved several dozen individuals who had consumed oysters from New Brunswick and subsequently experienced "DSP-like" symptoms, was never specifically identified.

In contrast, the cases of DSP in the greater Halifax area in 1990, caused by the consumption of contaminated mussels, were promptly confirmed by the application of a suite of previously unavailable chemical analytical and bioassay techniques. Expanded monitoring of shellfish products for DSP, particularly in the Scotia-Fundy fisheries region, has occasionally revealed the presence of toxins; however these compounds toxin levels tend to be low (usually <3 µg okadaic acid equivalents/g digestive gland) and toxicity is not persistent. In any case, even allowing for consideration of suspected DSP incidents, it seems evident that Canada is not subject to a current DSP crisis of major proportions.

Nevertheless, based upon knowledge of the confirmed DSP incident in Nova Scotia, the recurrent presence of suspect organisms, historical data on suspicious "DSP-like" episodes, and the seasonal occurrence of low levels of DSP toxins in Atlantic shellfish, Canadian authorities are in no position to be complacent. An area of particular concern is the potential spread of DSP toxin-producing organisms, and increases in bloom frequency, caused by the input of anthropogenic pollutants, global climatic changes, and/or the introduction of exotic toxic species. The effect of any of these events could be magnified by the simultaneous intensification of shellfish aquaculture and increased exploitation of wild stocks. The fact that vast sections of the Canadian coastline are closed to shellfish harvesting due to PSP or bacterial contamination may have masked the existence of a nascent DSP problem.

3. The paradox of Dinophysis toxicity and DSP in Canadian waters

If *Dinophysis* spp. are ubiquitous in coastal waters along both the Atlantic and Pacific coasts of Canada, why are incidents of DSP not common? Several explanations (not mutually exclusive) for this phenomenon were proposed: 1) inadequacies in detection methods and toxin monitoring programs has resulted in poor spatio-temporal coverage of potential DSP problems; 2) the frequency of DSP outbreaks may be correlated with the intensity of shellfish aquaculture and harvesting - much less in Canada than in Europe and Japan; 3) DSP toxicity in Canadian shellfish may be derived from certain *Prorocentrum* species, or perhaps other unknown planktonic organisms, rather than *Dinophysis* spp.; 4) *Dinophysis* blooms in Canadian waters may represent non-toxic populations, or may be only facultatively toxic under certain environmental conditions. In northern Europe, a few hundred *Dinophysis* cells per litre are apparently sufficient to toxify mussels to levels beyond the regulatory limit (20 µg okadaic acid equivalents/100g soft tissue), whereas in eastern Canada blooms of >50,000 cells L^{-1} have not resulted in substantial DSP toxin accumulation.

The first conclusive evidence of DSP toxins in phytoplankton from Canadian waters, was obtained from net tow samples from the Bay of Gaspé, Québec, in 1989. The presence of okadaic acid in samples in which *D. acuminata* and *D. norvegica* were the dominant species was confirmed by HPLC with fluorescence detection (HPLC-FD), immunoassay (UBE Industries) and ion-spray mass-spectrometry (ISP-MS). Cellular levels of okadaic acid were consistent with those previously found in Europe and Japan. However, the following year, DSP toxin was found in <10% of samples containing *Dinophysis* spp. from the Bay of Gaspé, and the amounts could not be quantitatively linked with the absolute or relative abundance of such species. Furthermore, massive blooms of *D. norvegica* and *D. acuminata* from Cardigan Bay, PEI did not reveal any DSP toxin by ISP-MS analysis, although a smaller population of the latter species did yield a positive response to the phosphatase inhibition assay.

Detailed chemical analysis of Nova Scotian mussels implicated in the first confirmed case of DSP in North America revealed substantial levels of DTX1 (maximum: 3µg/g digestive gland ≈ 60 µg/100g soft tissue). Based upon the co-occurrence of a *Dinophysis* bloom with the DSP intoxication episode, and the presence of thecal fragments attributable to *D. norvegica* in the mussel gut contents, there was strong circumstantial evidence that the culprit organism had been identified. Nevertheless, even through *Dinophysis* spp. was present in high relative abundance in subsequent net tow samples, no DSP toxin was detected in the phytoplankton. In contrast, in Mahone Bay, during an early summer bloom in 1991, the increase in *Dinophysis* cells numbers was found to anticipate the appearance of "DSP-like" symptoms in mouse bioassays. A later bloom of greater magnitude failed to exhibit this correlation. Does the poor correlation of DSP toxicity with *Dinophysis* occurrence indicate that it is not (always) responsible for toxic events?

Although the species was never in abundance in natural phytoplankton populations, an isolate of *P. lima* from net tow material obtained during the DSP episode produced significant quantities of both DTX1 and okadaic acid (in approximately equimolar ratio) in unialgal culture. Laboratory toxification experiments carried out in Belgium, which involved feeding a *P. lima* isolate rich in okadaic acid to mussels, have shown that after 10 days there was a gradual rise in the concentration of DTX1 in the mussel digestive gland. The results of a field program in Mahone Bay, Nova Scotia, the site of origin of the toxic mussels, have since shown that *P. lima* cells are commonly found growing epizootically on cultured mussels and are also present on the mesh socks used for mussel cultivation. Enumeration of *P. lima* is difficult due to the tendency of this species to be associated with an organic-rich substrate. A fundamental question remains: is *P. lima* a major cryptic source of DSP toxicity in Atlantic Canada?

4. Analytical methodologies for the detection of DSP toxins

In Canada, systematic monitoring for the presence of DSP toxins in shellfish is only carried out by the DFO Inspection laboratory (Halifax) in the Scotia-Fundy region. The mouse bioassay (I.P. injection) is used to screen samples from selected stations in Nova Scotia for the symptoms and death times characteristic of DSP intoxication. Since this bioassay is relatively insensitive and imprecise, suspect samples are assayed using the UBE DSP-Check immunodiagnostic kit, and when a positive response is indicated, HPLC-FD analysis is performed. Confirmatory analysis by ISP-MS, with the collaboration and assistance of the NRC analytical chemistry group, is occasionally necessary.

Unfortunately, reliable routine methods applied by regulatory laboratories to the detection of DSP toxins in Canadian shellfish are difficult to implement and are inadequate. As a consequence, the results are frequently inconclusive, if not contradictory. For example, concentrated extracts of mussels suspected of causing shellfish poisoning in eastern Nova Scotia in 1991, killed mice on I.P. injection and yielded a positive immunochemical response for DSP toxins, whereas HPLC-FD analysis failed to indicate the presence of either okadaic acid or DTX1. The mussels contained a red pigment (now attributed to a [non-toxic?] ciliate, ***Mesodinium rubrum***) and were reported to have a peppery taste. In some cases extracts, prepared according to the DSP protocol, particularly from Ship Harbour, Nova Scotia, appeared to exhibit a highly potent CNS-activity when injected into mice, resulting in rapid death. The situation was confounded by the appearance of DSP toxins (mostly DTX1) in mussel samples later in the summer, extract of these molluscs also caused mouse deaths. The chemical nature of the unidentified toxic factor (UTF) is not yet known. The UTF activity may be unrelated to DSP or other human intoxication syndromes - it may be entirely an artifact of the mouse bioassay, since I.P. injection is not a normal route of entry for such toxins.

Workshop participants identified major deficiencies in the techniques currently used in Canada for DSP toxin detection. The complexity of chemical analytical methods, particularly the HPLC-FD technique, and the need to refine and simplify clean-up procedures were frequently cited. The lack of adequate standards for certain DSP toxin analogues, specifically DTX1 and DTX3, hampers significantly the application of existing methods. The need for rapid technological transfer and training of laboratory personnel in the use of modified methods was also judged to be crucial.

5. Culturing DSP toxin-producing dinoflagellates

The Working Group was seriously divided on the appropriate level of effort which should be directed towards culturing DSP toxin-producing dinoflagellates, particularly *Dinophysis* spp. Several key research questions which could be addressed using cultured toxic organisms were considered: 1) life cycle studies; 2) toxin biosynthesis and metabolism; 3) ecophysiology and autecology of toxic species; 4) the role of associated bacteria in toxin production; 5) bulk toxin production through mass culturing; 6) genetics and molecular biology; and 7) toxin uptake and detoxification kinetics in marine food webs. All participants were in agreement that if it were practicable, the culture of *Dinophysis* spp. would be a worthwhile objective, as these species are responsible for the majority of DSP incidents. However, the intractability of *Dinophysis* spp. to sustained growth in monospecific culture was acknowledged. In spite of substantial efforts by European and Japanese colleagues in recent years, no viable *Dinophysis* culture capable of surviving serial transfers has been produced. Various hypotheses were advanced regarding the role of heterotrophic/phagotrophic nutritional modes, organic growth factors, chelator/trace metal ratios, and the fragility of the cells, to explain these failed attempts. Some participants were persuaded that further intensive research into *Dinophysis* culture could not be justified by cost/benefit analysis, given the scarcity of resources; others were less pessimistic, and concluded that successful culture of *Dinophysis* was not only attainable, but was also vital to DSP research programs.

Certain research goals involving *Dinophysis* spp., such as the tracking of bloom dynamics, the application of gene probes to natural populations, and even determining the specific toxicity per cell using cells isolate by micropipette, would not require culturing. However, viable cultures are obviously required for elucidating biosynthetic pathways, determining the kinetics of toxin production, establishing the role of bacteria in growth and toxin synthesis, and completing the life-history.

European researchers have used a DSP toxin producing clone of *P. lima* to determine and model the toxin uptake and detoxification kinetics. In the absence of *Dinophysis* cultures, workshop participants suggested that this approach be adopted using an available toxic strain of *P. lima*, such as the isolate from Mahone Bay. The requirement for high algal biomass for the preparation of DSP toxin standards, or of bulk toxins for experimental purposes, can be met through mass culture of *Prorocentrum* species currently in culture.

6. Research and regulatory recommendations

- Priority should be given to the development and implementation of reliable chemical analytical methods and assays to reduce dependence on the DSP mouse bioassay conventionally used in Canadian regulatory laboratories.

- Certified analytical standards for the major DSP toxins, particularly okadaic acid, DTX1, and DTX3, and reference materials containing DSP toxins in defined shellfish matrices, must be rapidly produced and readily available for calibration purposes.

- Canadian regulatory authorities, specifically National Health and Welfare, should be encouraged to set a realistic tolerance limit for DSP toxins in shellfish, either by accepting an existing limit presently adopted by EEC countries or Japan, or by conducting the necessary toxicological testing to derive a national standard.

- A retrospective survey of historical phytoplankton data on the spatial distribution of *Dinophysis* spp. in Canadian waters should be conducted.

- A broad-scale mapping of the geographical extent of DSP toxicity in commercially- and recreationally-exploited wild shellfish populations, analogous to the "Mussel Watch" program for marine pollutants in the United States, should be implemented in Canadian coastal waters.

- The source(s) of DSP toxins in shellfish in eastern Canada must be identified through field programs on the population dynamics of *Dinophysis* spp. and other putative DSP toxin-producing species.

- Efforts to determine alternative stages in the life history of toxic species, including possible resting stages should be expanded to include both cultured isolates and natural populations. The culture of *Dinophysis* spp. will be crucial to this endeavour.

- The biosynthetic pathways and kinetics of DSP toxin production should be established using available isolates of *Prorocentrum* spp.

- The possible introduction of exotic DSP-toxin producing species through the discharge of ship ballast water should be investigated, at least within the context of existing ballast water monitoring programs.

- In the absence of *Dinophysis* cultures, efforts should be made to exploit natural blooms, as well as DSP toxin-producing analogues, such as *Prorocentrum* spp., for investigating the transfer of DSP toxins in marine food webs.

- Since confirmed DSP incidents in Canada are relatively new and poorly understood phenomena, efforts should be made to develop and maintain international collaborative links and information exchanges (i.e. through ICES, IOC-SCOR, and national phycotoxin programs) with countries having more experience with DSP problems.

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Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #3

TRANSFER OF PHYCOTOXINS IN THE BENTHIC FOOD WEB

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In reviewing the mandate provided, the working group (WG) decided to augment the list of issues presented, and amend their order slightly. It is recognised that several of the points raised will be dealt with in greater detail by other working groups, but they are nevertheless recorded for completeness.

1. Transfer of toxins from the phytoplankton

The WG concluded that the transfer of all phycotoxins is for the most part within particles, that is, the toxin is transferred by feeding on the synthesising organism, or its resting stages, or other animal which has fed directly or indirectly on phytoplankton. While there is some evidence that toxins may be absorbed directly from solution, the rates and quantities are so low as to be irrelevant in any practical sense. There may be a possibility of ingestion of flocs, which contain toxins chelated onto the surface of flocs which may form in the vicinity of mussel socks or other specialised habitats. The retention of toxins in cysts and vector organisms may prolong the duration of a toxic incident beyond the phytoplankton bloom, and beyond the original geographic boundary of the bloom by transport inshore or offshore. There is particular concern that sediments may act as reservoirs for re-initiating blooms.

There is evidence of some capacity for discrimination of PSP by some plankton browsers, and some selective (avoidance) feeding. It is not clear that this feature leads to any useful control mechanisms or options.

There is some concern for the transfer and resuspension of toxic organisms from pseudofeces, and for the viability of cysts passed undigested through the guts of shellfish. This will be alluded to in the final section on control of shellfish transfers.

The WG noted particularly the concern over the identification of toxic and non-toxic strains of DSP-producing organisms

2. Transfer within Benthic food webs

Discussion concentrated on the transfer of toxins to animals other than direct plankton or filter feeders, and was characterised by an acknowledged ignorance of the food webs of benthic species in general and commercial invertebrates in particular. Food vectors are, in general not known although for some species, such as whales and lobsters, some deductions are possible. In all instances the toxin appears to be transferred in the food, and absorption direct from water is likely to be inconsequential. For the predator concerned, the toxin is merely part of a food item; only when it impacts humans in a manner related to public health, commerce, or newsworthy events, do we take notice.

The working group noted that concern was already being expressed about the capacity of lobsters and crabs to sequester toxins in the digestive glands, extending the known concerns for the sequestering of toxins by molluscan shellfish. The WG recommends that ethnic eating habits be considered since there is a reliance on non-traditional fishery products, such as whelks and moon snails which prey upon bivalves, and that consideration should be given to reviewing toxin concentrations in winkles, sea urchins, sea cucumbers, worms, and other species which may be the object of unregulated food fisheries.

Concern was expressed that recreational species such as ducks might be vulnerable to toxic shellfish.

The simple act of eating toxic organisms may not constitute uptake in a physiological sense. Shellfish may screen the toxic organism in the mantle cavity, on the labial palps, or in the gut. Toxins may remain predominately in the gut, and may not be digested and absorbed. Domoic acid may remain largely in the gut and digestive gland

and not enter the body in significant amounts.

The possibility that trace amounts found in other tissues may be associated solely with the hemolymph should be verified. Other toxins, being lipophilic, are clearly taken into the body and may be distributed in a selective fashion. The role of the hemolymph as transport and storage system should be elucidated.

3. Distribution in tissues

Typically the digestive gland will have high concentrations of all toxins studied, with smaller but possibly significant concentrations in other tissues. Typically the adductor muscles do not sequester the toxins. The possibility was discussed that the sequestering of PSP toxins in the siphons of soft-shell and butter clams may confer some selective advantage. Clearly lobsters and other species are able to detect toxins and there is evidence that some of the toxins are avoided. Notwithstanding their ability to identify and avoid toxin-containing prey, predators invariably accumulate toxins. The WG recommended that the distribution of toxins in local species be more thoroughly investigated.

The mechanisms by which the refractory toxins (PSP and DSP) are bound to the tissues is unknown. There was some speculation that release may be under some enzymatic control and that discovery of release mechanisms could allow the salvage of toxic shellfish for commercial use by maceration and treatment with appropriate enzymes. It was suggested that sequestering of toxins may be linked to the deposition of melanin. There is some evidence that PSP may be incorporated into the shell matrix.

4. Physiological effects

Discussion was limited to the effects in benthic invertebrate species and did not include any consideration of toxicology in vertebrates.

PSP is known to affect growth rates, heart rates, opening and closing of valves, of some bivalve species, and may have that effect while in solution. Prior history of exposure may increase sensitivity. There was discussion as to whether toxins in solution might adversely affect the growth and reproduction of bivalve larvae in culture conditions (hatcheries), and whether the operation of hatcheries for breeding, and culture of larvae, might be compromised during the course of a toxic bloom. This should be explored.

Domoic acid has so far shown no evidence of physiological effects when fed to marine invertebrates, although there is evidence that it is toxic to copepods when dissolved in sea water; copepods fed toxin-producing *Nitzschia* were unaffected. Lobsters and crabs are able to detect and normally avoid toxin-laden food. From a fisheries perspective this may be regarded as a positive effect. Clearly domoic acid, either the parent compound or some derivative, must have some physiological action in crustaceans since it can be used as an insecticide, but its lower toxicity may reflect the effects of being administered in an aqueous medium, or ingested. Its toxicity in vertebrates can be explained, but not in invertebrates. There was some discussion on whether the sequencing of *Nitzschia pungens* blooms could be explained by the domoic acid released from f. *multiseries* preventing f. *pungens* from blooming; and whether *Nitzschia* was in all other respects a nutritious alga.

While it is known that DSP will accumulate in tissues, the effect on consuming organisms is largely unknown. This is a wide-open field for enquiry. There was a suggestion that evidence of the effects of DSP may be available from countries where this toxin is more commonly reported than in Canada.

5. Elimination

There is no clear understanding of what prompts natural loss of toxins, nor how they are unbound from the tissues. It was generally felt that detoxification of PSP was a metabolic activity. ASP (domoic acid) appears to be very lightly bound, if at all, and, for the most part, may be simply flushed out. Clearance of the digestive gland takes longer than gut clearance, and the last remaining amounts may persist several days. The elimination of DSP is unclear, but its lipophilic nature suggests that it may be bound. There is some evidence for the presence of detoxifying enzymes. If such is the case, then the presence of a gene for a detoxifying enzyme could likely be manipulated for some sort of advantage. In sum, the chemistry and physiology of elimination are largely unknown.

There was some discussion about the 'magic' numbers: 80μg/100g for PSP and 20μg/g for domoic acid, and whether these truly reflected the appropriate limits for both acute and chronic exposure. The urgent need for control limits for DSP was noted.

Depuration is seen as a deliberate exploitation of known chemical and physiological factors to accelerate the natural elimination process for commercial or related purposes. Attempts to depurate PSP using ozone or other oxidants have been unsuccessful due to the PSP molecules being well sequestered in the cells. Depuration of domoic acid appears to have some potential for some species, such as bay scallops, which have a narrow harvest- or market window. Natural elimination rates and the potential for depuration of DSP are unknown. The difficulties of experimentation were discussed, particularly the problems of maintaining a continuous supply of algal cultures in quantities sufficient to obtain meaningful levels of contamination in adequate numbers of the target species. There is potential for the use of microspheres coated with okadaic acid, or of cultures of *Prorocentrum* for uptake and elimination studies of DSP.

On several occasions throughout the WG sessions there was discussion on the use of the term "concentration", whether shellfish actually concentrated the toxins under discussion. The conclusion reached was that there was no bio-concentration in the same sense that heavy metals may become bio-concentrated, and that the concentration in a predator would not likely exceed the concentration in the prey, but would clearly exceed the concentration free in the water. Reason suggests that in heavily feeding animals, where rate of uptake exceeded elimination rates, the concentration of toxin may temporarily exceed the concentration in the food. This might be checked relatively easily.

6. Surveillance

From the foregoing it is clear that some rethinking of the surveillance program is in order. Historically, there has been a shift in the nature of the agencies that were responsible for surveillance. Originally, both in Canada and the U.S., testing was done by the Health Departments, later the responsibility was passed to the Fisheries Departments, with the Health Departments setting the standards and establishing performance criteria. There now appears to be a trend to a further delegation of responsibility with the industry playing an increasingly more important role, and with the involvement of private-sector analysts. In some instances this appears to be by default due to cut backs in government agencies. The WG felt that this trend would continue with the private sector becoming increasingly responsible for product safety as well as product quality, and that close co-operation between industry and the research teams was essential.

There is still a significant improvement required, specifically in the provision of standards, and the establishment (where necessary) and review of official tolerance levels. There is a need for evaluation of toxin levels in species harvested in unregulated fisheries and products destined for ethnic or specialty markets in Canada or overseas. Examples given were: whelks, winkles, sea urchins, sea cucumbers. There was serious expression of concern for hitherto unrecorded toxins, and the need to maintain a world-wide watching brief.

As a final point, the group noted the potential for transfer of toxic strains of phytoplankton in normal shellfish commerce and particularly in the transfer of live shellfish for aquaculture. While this question is being addressed by other research teams, the WG felt it should also be identified here.

Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #4

TRANSFER OF PHYCOTOXINS IN THE PELAGIC FOOD WEB

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INTRODUCTION

Over the past decade, about 40 research articles on the interaction between phycotoxins and members of the marine pelagic food web have been published, a substantial increase from the 1-2 publications in the ten years (1967-1977) previous to the seminal work of A. White. The working group was convened in order to discuss the recent advances in the field. It was assigned the following questions: 1) What is the effect of algal toxicity on zooplankton? 2) Does algal toxicity have an impact on recruitment of commercial species? 3) Is algal toxicity transferred to adult fish species and what is its effect?

The working group held three meetings during the Workshop. During the second meeting, it divided into three subgroups, each of which addressed one of the above questions. To assist the subgroups, a list of the published research (see references) directly relevant to the subject of phycotoxins in the pelagic food web was compiled beforehand. Copies of approximately 80% of this material were available to working group members for reference during their discussions. At the third and final meeting, a representative of each subgroup presented a brief summary of its response to the assigned question, as well its recommendations for future study. A general discussion followed. Results of this effort are reported below.

1. Effects of phycotoxins on zooplankton

a. Microzooplankton

Some tintinnid species (e.g. *Favella* sp.) graze on and thrive in the presence of low or moderate concentrations of *Alexandrium tamarense*. Experimental studies have shown that, as concentrations of toxin increase, swimming behaviour and growth are affected and, at sufficiently high concentrations, exposure is lethal. Depending on the species of predator and toxin-producing prey, the harmful effects may result from ingestion of toxic cells or from contact with their exudate. The importance of microzooplankton as a vector for transfer of phycotoxin up the pelagic food web is not known.

b. Macrozooplankton

Approximately 15 research articles on toxic algae effects on copepods and euphausids have been published in the past 15 years. There is no indication that exposure to or ingestion of phycotoxins is lethal. Avoidance of toxic algae or rejection of captured cells has been observed, but these behaviours are highly dependent on the species of grazer and on the particular characteristics of its dinoflagellate prey. In general, zooplankton do accumulate toxins upon exposure, regardless of the level of feeding and therefore must be regarded as an important vector of phycotoxin transfer. The extent of transformation of the toxin composition by the grazer may also be species dependent.

2. Effects of phycotoxins on larval fish and impact on recruitment

In contrast to the response of invertebrate zooplankton, ingestion of PSP toxin is lethal to ichthyoplankton. Intoxification can occur by direct feeding on toxic cells by phytophagous larval stages or by feeding on zooplankton prey contaminated with toxin. The rate of mortality due to PSP poisoning depends on the fish species (its feeding mode and physiological sensitivity), the toxic cell concentration and the toxicity of the algal strain or of the contaminated prey.

The risk of exposure and subsequent deleterious effects on recruitment depends on the extent of correlation of larvae and toxic algae in space and time and on the toxin residence time in the plankton once the toxic bloom is over. The capelin population in the St. Lawrence Estuary, and spring and fall spawning herring in the southern Gulf of St. Lawrence are examples of stocks at greater risk to this source of mortality in eastern Canada. However, demonstration of actual negative impact on recruitment of any fish stock has been elusive.

3. Transfer of phycotoxins to higher trophic levels of the marine food web

a. Fish

PSP poisoning is lethal to finfish and is known to have caused herring kills in the Bay of Fundy. Transfer of toxin occurs by ingestion of contaminated zooplankton prey or, in the case of filter feeding fish, by direct ingestion of toxic algae. Residues of PSP toxin have been found in internal organs of mackerel. The working group was not aware of research on the extent of PSP toxin levels and PSP-related mortality in natural populations of other exploited fish species.

The same trophic mechanisms of transfer to fish apply to other phycotoxins. Recent research has shown domoic acid in high concentrations in the guts or tissues of anchovies from Monterey Bay, CA, and in trace concentrations in fish meal (possibly from sardines).

b. Marine mammals and sea birds

Phycotoxins have been implicated in several cases of mortality of marine mammals and sea birds. PSP contaminated mackerel were found in the stomachs of humpback whales found dead on Cape Cod in late 1987. The mortality of bottlenose dolphins along the east coast of the U.S. has been associated with feeding on menhaden contaminated with brevetoxins produced during a bloom of *Gymnodinium breve*. Domoic acid poisoning of Brandt's comorants and brown pelicans has been related to the ingestion of anchovies feeding on domoic acid producing *Pseudonitzschia australis* in Montery Bay, CA. Other cases involving manatees and Hawaiin monk seals were also cited.

4. Summary and recommendations

In general, crustaceans (e.g. copepods, lobsters) are much less affected by PSP ingestion than vertebrates, whose more sophisticated nervous systems are particularly vulnerable to the neuropathological effects of sodium channel blocking. There is a growing recognition in marine ecological research that phycotoxins are widespread in the marine environment and can be transferred via food web interactions (e.g. algae-crustaceans-fish) to higher trophic levels, where there can be serious deleterious impact. The following fourteen questions were recommended as research priorities. They reflect our rudimentary knowledge of the pathways of transfer and the specific effects they may have on marine populations. They are not listed in any particular order of importance.

1 . How do microzooplankton interact with toxic algae, with specific reference to their roles as grazers, toxin accumulators and links in the transfer of toxin to higher trophic levels?

2. What are the feeding and toxin accumulation responses of keyspecies of zooplankton?

3. What direct and indirect impacts do domoic-acid-producing algae have in the marine food web?

4. Do toxic blooms occur and are they important ecological phenomena in offshore and arctic waters?

5. Do dissolved extracellular toxins derived from toxic algae have an ecological impact in pelagic ecosystems (e.g., can they be absorbed by zooplankton and can they damage fish tissue)?

6. What are the sublethal effects of chronic exposure to phycotoxins in fish and marine mammals? What are suitable biochemical, physiological or behavioural indicators of sublethal effects?

7. Is it possible to transfer toxin to eggs or offspring ("horizontal" transfer)?

8. What are the depuration or elimination rates of phycotoxins in fish and zooplankton? What are the dispersal rates and residence times of phycotoxins in marine food webs?

9. In fish, can chronic exposure lead to toxic body levels?

10. Are fecal pellets an important vector of transport of toxin to the benthos?

11. Are there other, as yet unknown, algal species that produce toxins (e.g., species of Chaeotoceros or Heterosigma)?

12. To what extent are phycotoxins responsable for fish kills and mammal strandings? (At every such event, phycotoxin levels should be routinely measured).

13. What is the body load of PSP toxin and domoic acid in exploited species (e.g., herring, salmon, mackerel, herring roe) in areas and times when toxic blooms occur?

14. Does phycotoxin contaminated benthic preys have a harmful impact on groundfish stocks?

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Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #5

DETECTION AND QUANTIFICATION OF TOXIC ALGAE AND TOXINS

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INTRODUCTION

The Working Group was convened to provide the Phycotoxin Working Group with expert opinions on detection and quantification of toxic algae and algal toxins using the following general guidelines to help initiate the discussions:

1. Paralytic shellfish poisoning (PSP) toxin standards production: assessment of the situation

2. Other phycotoxin standards: needs

3. Need for inter-laboratory calibration

4. Development of new methods for the detection of toxins

5. New and rapid methods for identification of harmful algae.

The Working Group discussions are broken down into two major themes; instrument calibration standards and inter-laboratory calibration studies, and laboratory and field based assays and analyses. This report summarizes the results of the Working Group discussions and lists some recommendations for future action.

Instrument Calibration Standards and Inter-laboratory Calibration Studies

PSP toxin standards production - assessment of the situation.

Four groups and two private companies are involved, or at least claim to be involved, in the development of PSP toxin standards. The quality of the standards to be made available is questionable and members of the Working Group felt that there is a need to establish and/or define the criteria by which a standard is certified. General agreement on certification would not only allow for regulation of the quality of the standards produced, but it would also allow producers to differentiate between certified instrument calibration solutions and standards of lower quality. Lower quality standards are generally developed for use in biochemical studies and usually only include a statement of purity based on analysis by high performance liquid chromatography (HPLC) and/or mouse bioassay.

As part of the Marine Analytical Chemistry Standards Program (MACSP), the National Research Council of Canada (NRCC) Institute for Marine Biosciences (IMB) has recently completed a six month stability study on standard solutions of saxitoxin (STX), neosaxitoxin (NEO), and a mixture of gonyautoxins II and III (GTX-2 and GTX-3). Within the next year this group expects to release certified standard solutions of these four PSP toxins with the possible addition of decarbamoylsaxitoxin (dcSTX). Certification will include statements on both stability and purity. Purity will be determined by analyses using high performance liquid chromatography with fluorescence detection (HPLC-FD), capillary electrophoresis (CE) with ultraviolet detection, nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and capillary electrophoresis coupled with ionspray mass spectrometry (CE-MS). Absolute concentrations will be determined by NMR spectral comparison to a standard of known concentration (sucrose for example).

A consortium of laboratories in the European Community (EC) has been established to prepare PSP toxin instrument calibration solutions (Goenaga and Wagstaffe 1991). A French laboratory is evaluating the possibility of producing standards for gonyautoxins II and III (GTX-2 and GTX-3) (Lapeyre et al. 1991) and a Spanish laboratory is planning to produce STX, dcSTX, and gonyautoxins I and IV (GTX-1 and GTX-4). Release dates for the standards are currently under discussion within the EC laboratories involved. The methods used to certify or otherwise document the quality of the standards are presently undisclosed.

The United States Food and Drug Administration (US-FDA) currently supplies standard STX solutions for calibration of the Association of Official Analytical Chemists (AOAC) mouse bioassay method for analysis of PSP toxins (AOAC 1990), and it has also made available "standard" mixtures for inter-laboratory calibration (see below) and for method development (Lawrence and Ménard 1991). It was not known to members of the Working Group what was the status or expected release date for standard solutions of other PSP toxins by the US-FDA, nor was it known if the standards will be certified. It should be noted that because of a shortage of PSP toxin standards, Dr. J. Hungerford (US-FDA) recently resigned as the AOAC Associate Referee for the topic: *Paralytic Shellfish Poisons, LC Determination* (Hungerford 1992).

Dr. K. Oshima in Japan has made available "standards" of PSP toxins to many groups throughout the world. The absolute concentrations of the standards was determined by elemental nitrogen analysis and both HPLC-FD and NMR are used to establish purity. Although the standards are not rigorously certified, they have allowed many groups to establish and troubleshoot HPLC-FD analysis systems (Oshima 1989).

Two companies sell samples of PSP toxins of given concentrations. Calbiochem (P.O. Box 12087, San Diego, CA 92112-4180), which for many years has supplied STX, now lists NEO, GTX-1, GTX-2, GTX-3, and GTX-4 in their 1992 catalogue. Orders for the newly available toxins remain on back order so availability is questionable. Chiral Corporation (1110 Brickell Avenue, Suite 407, Miami, FL 33131) also lists STX, NEO, GTX-1, GTX-2, GTX-3, and GTX-4 as available products. As is the case for Calbiochem, orders for these toxins remain unfilled.

PSP toxin inter-laboratory calibration studies

Some members of the Working Group participated in the US-FDA/AOAC pre-collaborative study for HPLC-FD analysis of PSP toxins using the method developed by Sullivan and Wekell (1987). It appears that only one-half to one-third of the laboratories who undertook the exercise actually completed the study (Wekell 1991). Some laboratories consumed their entire supply of PSP "standard" setting up and troubleshooting their HPLC equipment and these groups were unable to obtain enough of the "standard" mixture to complete the study.

Working Group members from the EC stated that eighteen laboratories were conducting a STX inter-laboratory collaborative study to ascertain whether or not all labs could perform the analysis satisfactorily. The material for analysis is STX spiked into shellfish tissue samples and most labs were using the HPLC-FD method developed by Oshima et al. (1988) for the work. It was felt that Oshima's method might be the easiest to maintain on a regular basis and that it is readily amenable to automation. Because of their proclivity for Oshima's method, European Working Group members stated a preference for PSP instrument calibration solutions that were available as separate mixtures of the A (STX, dcSTX, NEO), B (GTX-1 to -4, B-1, B-2) and C (C-1 to -4) groups of PSP toxins.

Diarrhetic shellfish poisoning (DSP) toxin standards: assessment of the situation

Although many companies sell okadaic acid (OA), a certified instrument calibration standard for OA, the dinophysistoxins (DTX's), the pectenotoxins, and/or the yessotoxins is not presently available. Some groups are using commercially available OA as a standard for HPLC analysis by the 9-anthryldiazomethane (ADAM) derivatization method (Lee et al. 1987) and, by assuming the same response factors for DTX-1, DTX-2 and DTX-3, these toxins are also being quantified. The NRCC will soon be releasing a certified OA reference material (called MUS-2).

DSP toxin inter-laboratory calibration studies

Although the HPLC fluorometric method for the determination of okadaic acid using bromoacetylpyrene as the labeling reagent has been suggested for collaborative study (Hungerford 1992), to date, no inter-laboratory collaboration studies have been reported for DSP toxins. The EC countries are currently conducting an inter-laboratory study on DSP toxin analysis using the ADAM derivatization method.

Amnesic shellfish poisoning (ASP) toxin standards

The NRCC has a certified instrument calibration solution for domoic acid (called DACS-1) and also a certified domoic acid (DA) mussel reference material (called MUS-1) (Hardstaff et al. 1990). The US-FDA also has a DA instrument calibration solution available.

ASP toxin inter-laboratory calibration

The methods of choice for DA analysis are HPLC with ultraviolet (UV) detection at 242 nm (Quilliam et al. 1989; Lawrence et al. 1989) or HPLC with precolumn derivatization and fluorescence detection (Pocklington et al. 1990). An inter-laboratory calibration study for DA analysis by HPLC with UV detection was recently published (Lawrence et al. 1991).

Other toxin standards

Other phycotoxins were not specifically discussed by the Working Group.

Laboratory and field based analyses and assays

Toxins

The members of the Working Group felt that it was important to describe and discuss the differences between laboratory and field based analyses and assays. Laboratory based assays and analyses are performed by highly trained personnel at regional and or national locations. Field based methods are performed by minimally trained personnel with simple or self-contained portable equipment. (Note, the equipment may be sophisticated, for example, similar to a portable pH meter, but it would not require extensive knowledge of how the instrument works for it to be used effectively).

Field based phycotoxin assays and analyses can be further subdivided into two groups; those performed by the actual shellfish producer, and those performed by site based facilities servicing a restricted geographical area. Field tests should be rapid, robust, simple to use, cheap and, at least, semi-quantitative. For example, there is a need for field based methods capable of detecting PSP toxin contaminated shellfish, particularly for the West coast shellfish industry and for the East coast offshore scallop fishery (jobs and economic development). Field based methods could be used to screen for PSP contaminated samples and could conceivably cut down on the number of samples that would be need to be analyzed by expensive and time consuming laboratory based methods. Site based laboratories could utilize equipment that is not readily portable, such as a test in the development stage to distinguish between toxic and non-toxic domoic acid containing mussels by ion mobility spectrometry (Elias and Lawrence 1990).

Because of the selectivity of antibodies for the target analyte, immunochemical methods appear to offer the most potential as field based tests. The NRCC Institute for Marine Biosciences has an ongoing program to develop monoclonal antibodies initially for the four most abundant of the PSP toxins (STX, dcSTX, NEO, and a GTX-2 + GTX-3 mixture). The objective is a field test based on mixed monoclonal antibodies. Monoclonal antibodies offer the advantage of continuous supply but they can be less sensitive that polyclonal based methods. Monoclonal antibodies have been developed for STX (Hack et al. 1990; Huot et al. 1989) and polyclonal antibodies for NEO have been described by Chu et al. (1992). An immunochemical test kit for STX based on a polyclonal antibody was developed by Institut Armand-Frappier (531 Boulevard des Prairies, Laval, PQ), but field testing by DFO was disappointing. Polyclonal stick tests for STX have also been developed by Pagé (personal communication) at Laval University and Schneider et al. (1991, Usleber et al. 1991) in Munich.

In order to try to establish priorities, a general discussion was held on the relative importance of developing immunochemical methods for PSP toxins vs other shellfish toxins. Although PSP toxin contaminated shellfish may be a more widespread problem, it was agreed that all toxins need to be monitored in the interests of public health and support of the shellfish industry. People not only get sick from shellfish poisoning, but jobs are also at stake, whether the toxins kill people (PSP and ASP toxins) or not (DSP toxins).

Immunochemical methods for detection of DSP toxins have been developed by Usagawa et al. (1989) and Rougier Bio-Tech Ltd. (8480 boul. St-Laurent, Montréal, PQ). Rougier Bio-Tech has produced an enzyme-linked immunosorbent assay (ELISA) kit for quantification of OA that, at the time of the Working Group meeting, was being distributed free for evaluation. Rougier Bio-Tech is now working on development of an immunochemical based OA stick test.

Laboratory based analysis methods are well established for most of the common marine phycotoxins. The ASP toxin, DA, is currently monitored by DFO using a HPLC based method with UV detection (Lawrence et al. 1989). Since the outbreak of domoic acid poisoning of pelicans in California (Fritz et al. 1992) and the observation of high levels of domoic acid in razor clams in Washington State, domoic acid monitoring programs have been set up by the US-FDA in specific areas on the West coast of the United States.

A fluorescence based HPLC method for OA is well established (Lee et al. 1987; Pleasance et al. 1990), however, monitoring for DSP toxin contaminated shellfish by instrumental methods is not routine at this time. The extraction method for the DSP toxin HPLC analysis method is very lengthy and there is a need to improve and speed up the entire process. Currently, DSP toxin regulation in Canada is performed by DFO using the mouse bioassay.

Sensitive instrumental methods for PSP toxin analysis are well developed, particularly HPLC (Sullivan and Wekell 1987; Oshima 1988) and more recently CE based methods (Pleasance et al. 1992; Thibault et al. 1991; Wright et al. 1989). The members of the Working Group agreed that a CE method using post-capillary oxidation with fluorescence detection could be the analysis method of the future for PSP toxins. Despite the general acceptance instrumental methods for PSP toxin analysis, it is doubtful that the mouse bioassay will be replaced by an instrumental method until standards are available for all of the naturally occurring PSP toxins, and the relative toxicities of the PSP toxins are rigorously established. (The EC has already postponed replacing the mouse bioassay for just this reason.)

Tissue culture bioassays may offer an alternative to the mouse animal bioassay for quantitation of PSP toxins (Kogure et al. 1988; Kogure et al. 1989; Jellett et al. 1992), and possibly other naturally occurring neurotoxins. The mouse neuroblastoma cell assay for PSP toxins as developed by DFO has proven to be flexible, reliable, sensitive (0.01 ug/mL minimum detection limit for STX), and inexpensive (Jellett et al. 1992). It provides essentially generic detection of PSP toxins, and, like the mouse bioassay, it has a reduced requirement for multiple toxin standards. In a side by side comparison performed by DFO, results from the tissue culture assay proved to be identical to those from the mouse bioassay (Jellett et al. 1992). The cost of the bioassay is approximately $3 per sample and one person can perform around 100 bioassays per day. This can be compared to the mouse bioassay where a single person can do 30-40 samples per day. PSP bioassays such as the lobster nerve preparation, mollusc nerves, and other cell based methods for detecting sodium channel blockers were only briefly discussed by the Working Group.

Toxic algae

At the present time, it is known that approximately ten species of marine algae occurring in Canadian marine waters have the potential to cause harmful effects on both marine organisms and human consumers. Data from monitoring of harmful marine algae can be used to provide a basis for understanding whether toxic events are normal or are related to exceptional meteorological events or anthropogenic activity. The Working Group discussed one recent development in detection methods for harmful marine algae.

A poster by Bates et al., displayed at the present Workshop, described the development of an immunofluorescence method to detect and differentiate between the diatoms ***Nitzschia pungens*** f. ***multiseries*** (the domoic acid producing form) and N. ***pungens*** f. ***pungens*** (the non-toxic form). Antisera against N. pungens f. ***multiseries*** did not cross-react with ***N. pungens*** f. ***pungens***, and antisera against ***N. pungens*** f. ***pungens*** did not cross-react with ***N. pungens*** f. ***multiseries***, even at high concentration. There were no cross-reactions of the ***N. pungens*** f. ***pungens*** antiserum with the six other phytoplankton species tested (***N. pseudodelicatissima***, ***Cylindrotheca closterium***, ***Bacillaria*** sp., ***Chaetoceros*** sp., ***Gyrosigma*** sp., and an unidentified chlorophyte). Immunofluorescence shows great promise as a technique to distinguish between and to quantify the two forms of ***N. pungens***.

RECOMMENDATIONS

1. Establish and/or define criteria by which an instrument calibration standard is certified.

2. Certified instrument calibration standards should be developed for all of the naturally occurring PSP toxins.

3. The relative toxicities of the PSP toxins should be rigorously established.

4. Any inter-laboratory calibration studies should be undertaken jointly with the AOAC.

5. Further research should be directed towards developing immunochemical methods for detection and quantification of toxic algae and toxins.

6. A future workshop should address standardization of methodology for cultivating toxic algae.

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Third Canadian Workshop on Harmful Marine Algae

Report of Working Group #6

MONITORING TOWARD YEAR 2000

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INTRODUCTION

At this workshop, the various working groups were organized into four categories. Working Group 6, subtitled 'Monitoring towards the year 2000', was the only working group included in the category 'Monitoring and Prediction'. This group was asked to consider four questions:

1) Are monitoring progams useful?
2) What do we need to predict blooms of harmful algal species?
3) Is there a need for predictive models?

4) The water ballast concern: is deballasting an underestimated problem in Canadian waters?

It was not clear from this whether monitoring of shellfish toxicity was to be considered by the working group or if the discussion was to be confined to phytoplankton only. Further, the last three questions imply a very broad definition of the term 'monitoring' and a strong inclination to view monitoring and prediction from the research perspective. The latter bias is understandable since the questions were framed by research scientists. We suggest below, however, that monitoring *per se* is an applied or operational science which requires active research support and may yield valuable scientific data as a byproduct, but whose objectives should not be confused with those of a research project.

The tendency to view monitoring from a broad, research-oriented perspective was also apparent in a document entitled *Overview of Canadian Phytoplankton Monitoring Programs* which was prepared for presentation at this same workshop. This document also did not consider shellfish toxicity monitoring but recognized no less than four types of phytoplankton monitoring:

1) Long-term trend monitoring,
2) Monitoring in support of research programs,
3) Monitoring of opportunity, and
4) Phytoplankton watch monitoring.

It became apparent during discussions that a large proportion of the working group participants defined "monitoring" in a relatively restricted way, that is, largely as "phytoplankton watch monitoring" and the associated monitoring of shellfish toxicity. These people tended to be directly involved at all levels in the actual delivery of operational monitoring programs and did not seem to consider so-called "long-term trend monitoring" or "predictive models" as directly relevant to such activities. It was clear from this that the term "monitoring" needed to be more clearly defined. In the following, we first consider what is meant by the term "monitoring" and what benefits can be expected from this monitoring. It is interesting to note that a group of Massachussetts fishermen presented a brief to the recent Fifth International Conference on Toxic Marine Phytoplankton outlining what they felt should come out of that conference. This group essentially wanted to know what was needed to make harvesting safe. They wished that toxins and toxin producers could be quantified and identified, that a 1 to 2 week warning could be provided for impending toxic events, that a management plan was in place to deal with such toxic events and that public education and responsible media coverage would give consumers a proper perspective on the safety of seafood products. From this it is clear that producers also view monitoring from a practical perspective. We also consider what activities and time scales are encompassed by the term monitoring, who are the monitors and who their clients, and what is the role of research in this process. We hope that the answers to most of the questions posed above will then be fairly clear.

Definition of Monitoring

According to the Shorter Oxford Dictionary, the verb 'to monitor' means to remind or to give warning. 'Phytoplankton watch monitoring' is carried out by DFO Inspection Services Branch and other parties in certain regions of Canada (in this case region refers to a DFO administrative district). There is considerable interregional diversity in these programs as described in the *Overview of Canadian Phytoplankton Monotoring Programs*. 'Phytoplankton watch monitoring' is meant to give an early warning of impending toxic events and clearly satisfies the dictionary definition of monitoring. Phycotoxin problems are also dealt with by monitoring toxin levels in shellfish and other organisms of commercial interest. Although these monitoring activities also serve to alert the industry and may alleviate some of the economic problems associated with toxic algae, the primary purpose of this type of monitoring, from the regulator's point of view, is the protection of public health. Other harmful algae exist, which, although not toxic to humans, can cause immense economic damage to the aquaculture industry. At present, monitoring of this type of organism in Canada is the responsibility of the industry or other interested parties. In any case, we wish to define all such monitoring of algae, whether toxic or otherwise harmful, and of phycotoxins, whether in phytoplankton, shellfish or other organisms, as *operational monitoring*. Thus defined, operational monitoring is undertaken for short-term public health and economic purposes only. Operational monitoring can produce a large amount of data which is valuable for scientific purposes, but this is not its fundamental objective. When feasible, operational monitoring should be carried on in such a way as to maximize the utility of such byproduct data. Operational monitoring does, however, require a great deal of practical scientific research support and advice (see below "Research Programs in Support of Operational Monitoring". We feel that the only correct use of the term "monitoring" is in the operational monitoring sense.

Research Programs in Support of Operational Monitoring

As can be seen from the preceding heading, we have inverted the second definition of monitoring given in the *Overview of Canadian Phytoplankton Monitoring Programs* to reflect what we believe to be the true priorities. These research programs should deal with such questions as what harmful or toxic algal species and toxins are to be found in a given region? How toxic algal numbers and toxicity vary in space and time? What biological and physicochemical factors govern this variance? How effective operational monitoring can be ensured in the presence of such variance? What factors control the uptake and depuration of toxins by shellfish and other organisms? etc. In addition to providing feedback and quality control for operational monitoring programs, science should also provide advice and recommend procedures at a very practical level; this includes the provision of usable taxonomic keys, field manuals and training in phytoplankton identification and enumeration, practical microscopy, field sampling procedures, record keeping, etc.

Many different organizations carry out a wide variety of operational monitoring programs. By adopting appropriate and relatively standardized techniques, it should be possible to ensure the effectiveness of operational monitoring while protecting the integrity of its valuable byproduct data. Operational monitoring programs produce large amounts of such data which are of value to research projects which aim to provide various measures of predictability for harmful algal blooms. If possible, operational monitoring programs should gather as much hydrographic and other information as practical; for example, top and bottom temperatures would indicate whether the water column is stratified or not. Other samples, especially for phytoplankton toxicity measurements, but also nutrients and chlorophyll would be of great use. Arrangements should be made with the research project managers who are interested in such samples for their analysis and interpretation.

The third category of monitoring given in the *Overview of Canadian Phytoplankton Monitoring Programs* ('Monitoring of Opportunity') can easily be included in this section; this definition of monitoring was apparently mooted both to remind us to look beyond the usual inshore sampling sites for potential harmful algal problems and also to take advantage of the opportunities to sample the offshore which are presented by the many cruises which are carried out for other primary purposes. We agree with this and would go further, suggesting that it is a role of researchers to consider harmful algal problems throughout the world, both for comparative purposes to help in understanding local problems and to be aware of newly discovered harmful algae and novel outbreaks elsewhere.

It is important to note that most monitoring programs are really designed to detect the presence of toxins in inshore areas; this is largely due to the expense and logistical difficulties encountered in offshore sampling. However, important harmful algal blooms such as those associated with paralytic shellfish poisoning can be largely offshore phenomena which are entrained in coastal currents and may impinge on the inshore at various locations causing a rapid toxification of shellfish. In such cases, it is important to acquire a sufficient understanding of these blooms and their associated hydrography to be able to identify a group of key indicator stations which will provide as early a warning as possible of such toxic blooms coming onshore. It would be useful to study the possible role of remote sensing in tracking such blooms; this is already being investigated in the Gulf of Maine and may provide important insights into the spread of paralytic shellfish poisoning in the Gulf of St. Lawrence. It should be remembered, however, that such blooms can also be generated in situ within a given inshore area. Paralytic shellfish poisoning toxins also appear in offshore populations of shellfish such as scallops on George's Bank and carnivorous gastropods in the Gulf of St. Lawrence and elsewhere. Also, the sporadic outbreaks of paralytic shellfish poisoning recently observed in Newfoundland require study in order to devise effective monitoring procedures.

Utility of Monitoring

We may now consider the first question put to this working group. It is clear that operational monitoring programs have been of great utility in many DFO regions of Canada and in other parts of the world, such as the Gulf of Maine. Paralytic shellfish poisoning incidents have largely been avoided by monitoring key stations for the presence of species of *Alexandrium* in the water column and of toxicity in shellfish. A fine example of the usefulness of such programs is provided by the toxic blooms of *Nitzschia pungens* f. *multiseries* which occurred in New London, Malpeque and Cascumpec Bays in northwestern Prince Edward Island in October 1991. The latter two bays had not previously had such blooms and the timing, magnitude and toxicity of the bloom in New London Bay was unprecedented. Conditions were such that the growth of the toxic organism and feeding by mussels were at maximal levels which resulted in very rapid toxification of the shellfish. Additionally, American oysters were shown to accumulate domoic acid in the field for the first time. The phytoplankton and shellfish monitoring programs operated by the DFO Inspection Services Branch were nevertheless able to provide an early warning of these events sufficient to prevent any contaminated shellfish from reaching consumers, avoiding both human health problems and the damage to the fishing industry which would result from such items reaching the market. The latter benefit is particularly important since experience has shown that many sectors of the fishing and aquaculture industries can be harmed by the publicity consequent to an actual or rumoured toxic shellfish event. This was borne out by the discovery in late 1991 of domoic acid in the marine food web on the west coast of the USA; this resulted in the closing of many lucrative Japanese markets to US fish products and severe financial losses to the industry.

Even in the case of domoic acid, however, there are complexities for operational monitoring. One is the problem of false positives which could result from phytoplankton monitoring because of the existence of a morphologically identical (in the light microscope) but non-toxic form of the organism which produces most of the domoic acid locally. The discovery of a high level domoic acid producer in California also underlines the possibility of false negative conclusions from phytoplankton monitoring; we simply do not know the identities of all the domoic acid producers in the world. Under present conditions, the safest approach to preventing domoic acid problems would be to have monitoring programs which utilized existing sensitive analytical assays for domoic acid to back up phytoplankton observations and to support research programs which actively search for further domoic acid producers. Additionally, we do not know enough of the uptake and depuration characteristics for domoic acid for most shellfish or their tissues. It is known that low level domoic acid producers exist and it is possible that chronic exposure to such sources could result in the accumulation of dangerous quantities of the toxin in certain tissues of organisms which depurate this substance very slowly. Much further work must be done to improve operational monitoring before we can feel safe from domoic acid events.

It is clear from the foregoing that operational monitoring programs can be highly useful. However, the necessity to improve and extend such monitoring should not be forgotten because of a few successes. A particular problem on the Canadian east coast is that of diarrhetic shellfish poisoning. Research has shown that the organisms responsible for the suite of toxins which produce these toxic effects are present in abundance in local waters. These organisms are seldom toxic, although toxic events have been recorded. We not only need to identify those circumstances in which these organisms become toxic, but must also develop or encourage the development of sensitive, practical analytical techniques for the quantitative detection of the toxins. Further, unknown toxins have been shown to exist in local waters and these must be identified and investigated. An example of this is the unkown toxic factor present in shellfish from Ship Harbour, NS. This unknown toxic factor was detected by mouse bioassay and this points out the necessity to continue this form of toxicity monitoring as a broad screening technique for such unkown problems. Other species of algae known to have been harmful in other parts of the world are also known to be present in Canadian waters. Examples of these are *Gyrodinium aureolum* and various species of *Chrysochromulina*. *Heterosigma akashiwo* has caused great harm to the salmon aquaculture industry on the west coast of Canada, but not on the east coast where it is nevertheless present. Such organisms must be taken into account by research programs in aid of operational monitoring so that, in case such a harmful bloom should develop, it could be rapidly identified from live and preserved samples and ameliorative measures (if any) taken. We also must not forget the freshwater environment; the cause of a major fish-kill incident at an Atlantic salmon enhancement project at Mooney's Pond (PEI) has not yet been identified, but large numbers of green algae and possibly cyanobacteria were present at the time which suggests that an involvement of toxins such as microcystins cannot be ruled out.

Prediction of Harmful Algal Blooms

The second and third questions put to the working group deal with prediction of harmful algal blooms on short and long time scales. On the short time scale, that is, the few weeks normally associated with a major bloom, prediction of bloom development should be possible provided the mechanisms governing that particular bloom are understood sufficiently well. Thus, in the case of domoic acid producing blooms in eastern Prince Edward Island, very large toxic events in the fall are associated with long dry periods in the summer, followed by heavy fall rains which are succeeded by an extended period of calm weather. The eventual size and toxicity of a given bloom is governed by meteorologically forced nutrient inputs and blooms may be readily dispersed to sea by strong north winds. The development of toxicity in a bloom which has been initiated in this system can be predicted fairly well such that a 2 week warning of an impending closure of mussel harvesting can be provided under ideal conditions. Such prediction requires a strong understanding of the physical, chemical and hydrographic factors governing the system and the detection of the bloom in its initial stages. This latter can be provided by a sound operational phytoplankton monitoring program. Similarly, the time when a developing paralytic shellfish poisoning bloom in the lower estuary of tha St. Lawrence will affect downstream sites can be predicted fairly accurately.

However, both these systems are characterized by a very large degree of interannual variablility such that in some years there is no bloom at all or a bloom of a non-toxic organism will occur instead. Such great interannual variability is probably associated with the life histories of the relevant organisms and the factors governing the species succession of phytoplankton; both subjects require a great deal of further study before we can hope to acquire an ability to predict toxic blooms on the time scale of more than a few weeks. During the working group meetings, Drs. Mann and Blasco described earlier studies of long-term ecosystem variability and prediction and suggested that the best we can do at the moment is to predict what groups of organisms might occur at particular times during the season, but that the prediction of the timing of the appearance or population size of a particular species of a given group, while perhaps a hope for the future, is presently a remote prospect.

Therefore, predictive models, at least for the long-term, are presently of doubtful utility. However, the subject should not be ignored because it is difficult. It may be useful to appoint a study group to consider this problem and the associated problem of the management of the data bases which are coming out of the ongoing work on phycotoxins in Canada. These data could have many future uses if the programs were to be carried on long enough and may be of service in problems of climate, toxic contaminants etc. Such a study group could formulate hypotheses to test and conduct the multivariate and other analyses these data sets require. A program such as this may be a useful contribution to the international harmful algal blooms programs which are currently being developed by ICES and IOC.

The subject of predictive models is closely related to the so-called 'long-term trend monitoring' listed in the *Overview of Canadian Phytoplankton Monitoring Programs*. One of the most important hypotheses concerning harmful algal blooms is that their frequency, diversity, geographic range and toxicity are increasing due to anthropogenic alterations of the coastal marine habitat. This hypothesis has been supported by the few long-term data sets which exist for northern Europe where the concentrations and ratios of various important nutrients for phytoplankton growth have been observably altered over the last century. A corollary of this hypothesis is that harmful algal blooms may serve as indicators of coastal habitat degradation. In any case, it has been suggested at meetings of the Phycotoxins Working Group of DFO and elsewhere that a number of long-term monitoring stations should be set up to identify trends in physicochemical and biological variables over a span of at least several decades. If such a program was to become a reality, it would have to be instituted and managed at a level much higher than that of a normal project. Consideration should be given to the appointment of a study group to investigate the feasiblity and necessity of such long-term monitoring stations.

The Ballast Water Concern

The fourth question referred to the working group dealt with the subject of the role of ship's ballast water as a means of spreading species of harmful algae. This topic was considered at a DFO workshop last year, the proceedings of which have recently been published. That document contains a set of recommendations which cannot be improved upon here. We were asked to consider whether the ballast water problem is being underestimated in Canada. The problem seems to be taken very seriously. In addition to the workshop referred to above there is a strong program on this problem ongoing at the Maurice Lamontagne Institute. Additionally, there is at least one DFO subvention grant program on ballast water. The resources available for ballast water studies may or may not be ideal, but the problem has not been underestimated or unrecognized. It was suggested that a complete species list for Canadian waters would provide a tool which would help in the identification of exotic species when they are encountered. Concern was also expressed that the practice of relaying shellstock in different areas could also lead to the spread of harmful algae.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS AND RECOMMENDATIONS

The Third Canadian Workshop on Marine Harmful Algae allowed over 80 Canadian and foreign participants to exchange information on the latest developments concerning the subject of harmful marine algae in Canadian waters. Besides oral and poster presentations, six Working Groups were set up for general discussion on the following themes: (1) Origin and Propagation of Harmful Algae, (2) Transfer of Phycotoxins in the Food Web, (3) Detection and Quantification of Toxic Algae and Toxins, and (4) Monitoring and Prediction of Harmful Events. For each theme, a list of specific topics or questions for discussion was identified by the Scientific Committee on the basis of their potential national interest. Specifically, the mandate given to each group was to provide insightful opinions concerning the above mentioned subjects and to recommend the course of action for future research.

Following is a brief summary (unprioritized) of the most important recommendations contained in the six Working Group Reports which were regrouped according to the general themes mentioned above. For more specific recommendations, the reader should consult the different Working Groups Reports included in these Proceedings. The Scientific Committee also thought it useful to emphasize (*) the recommendations that particularly concern the immediate public safety (potential fatality).

(1) ORIGIN AND PROPAGATION OF HARMFUL ALGAE

* 1. Given the potential threat to public safety, determine the sources and locations (inshore/offshore) of domoic acid on both, the east and west Coasts of Canada;

2. Given the potential threat to public health and possible damage to the shellfish industry, determine the sources of DSP toxins in shellfish in eastern Canada and elucidate the circumstances in which *Dinophysis* spp., the potentially more important source, may become toxic;

3. Given our almost complete lack of data and the possible development of a limited commercial fishery, examine the potential occurrence of harmful algae and, in particular, phycotoxins in Arctic marine waters;

(2) TRANSFER OF PHYCOTOXINS IN THE FOOD WEB

* 4. Given the potential for accumulation in marine organisms, investigate more thoroughly pathways of phycotoxin transfer through the food web and the distribution of toxins (ASP, DSP, PSP) in the different organs and tissues of affected species;

* 5. Given increasing activity and diversification of the fishing industry, and the associated risks for human health, determine toxin levels in species harvested in unregulated fisheries and products destined for ethnic or specialty markets in Canada or abroad;

6. Given the importance for the fishing and aquaculture industries, improve our understanding of the chemistry and physiology of uptake and elimination of phycotoxins (PSP, DSP, ASP) in commercial species;

7. Given possible impact on recruitment of commercial species, investigate feeding and toxin accumulation responses of key planktonic species;

8. Given the greater phycotoxin sensitivity of the more sophisticated nervous systems in fish and marine mammals, determine sublethal effects of chronic exposure to PSP and DSP;

9. Given the prevalence and persistence of domoic acid in many commercially and recreationally harvested marine species (e.g. domoic acid detected in home-canned razor clams from as early as 1985 in Washington), determine the ecotoxicological potential impact of sporadically high versus persistently low exposure levels to domoic acid on selected marine species, and humans;

(3) DETECTION AND QUANTIFICATION OF TOXIC ALGAE AND TOXINS

10. Given increasing needs for control and monitoring, certified instrument calibration standards should be developed for all of the naturally occurring PSP, DSP and ASP toxins, and the relative toxicity of each toxin should be established;

11. Given the multiple sources of standards, establish and define criteria by which an instrument-calibration standard is certified;

12. Given the need for intercomparison of data, phycotoxin extraction and analytical methods should be standardized. In particular, the inspection laboratories should be fully equipped to perform these analyses using a standard protocol;

13. Given increasing analytical demand for control and monitoring purposes, there is a need to refine and simplify the chemical analytical methods, particularly the HPLC-FD technique, and clean-up procedures for DSP toxin detection;

14. Given the potential threat to public health and possible damage to the fishing and aquaculture industries, there is an urgent need to establish official control limits for DSP;

15. Given the importance to detect rapidly and accurately toxic algae and phycotoxin levels in commercially and recreationnally harvested species and to reduce dependence on mouse bioassay, further research should be directed towards developing immunochemical methods for detection and quantification of toxic algae and toxins;

16. Given increasing analytical demand for control and monitoring purposes, there is a urgent need for rapid technological transfer and training of laboratory personnel in the use of the standardized analytical methods;

(4) MONITORING AND PREDICTION OF HARMFUL EVENTS

*17. Given increased potential of domoic acid intoxication, intensify domoic acid monitoring of commercially important shellfish, crustaceans and finfish along the Pacific Coast of Canada;

18. Given potential importance and utility of monitoring programs for the public safety and the industry, back up operational phytoplankton monitoring programs with existing sensitive analytical assays for ASP toxins;

19. Given its importance to public health and safety, examine the possibility of extending the Mussel Watch Program to DSP and ASP;

20. Given the need for prediction, increase studies of life histories of relevant organisms and the factors governing the species succession of phytoplankton in order to acquire an ability to predict toxic blooms;

21. Given the increasing importance of monitoring programs in Canada and Worldwide, a study group should be established to consider the management of the data bases which are coming out of the ongoing work on phycotoxins in Canada and to investigate the feasibility and necessity of long-term monitoring stations;

22. Given increasing importance of environmental and water ballast monitoring programs, a complete list of known toxic and harmful algae should be

established.

The series of Canadian Workshops on Harmful Marine Algae represents a unique opportunity for Canadian and an increasing number of foreign participants to discuss and exchange ideas on the various aspects of the problem of harmful and toxic algae in the marine environment. These workshops permit a rapid diffusion of new information and are favouring the establishment of inter-laboratory collaboration. In spite of the strong national focus of the Canadian Workshops, the participation of scientists from other countries facing similar problems is very useful, if not essential given the international nature of many problems, and should be encouraged. A good example is the domoic acid problem, for which closely linked efforts are needed in Canada and United States to carry out the required research to determine the sources of domoic acid, to understand the physical and biological mechanisms that lead to domoic acid accumulation in marine animals, to standardize detection methods, and to establish efficients monitoring programs. The importance of developing and maintaining international collaboration to exchange information with countries having more experience with certain problems newly occurring in Canada (eg. DSP) was stressed by several participants to the Mont-Joli Workshop. In that context, it is equally important to carry out intercalibration exercises and maintain collaborative links through international organisations such as ICES, IOC-SCOR, AOAC, just to name a few, for intercomparison purpose.

In conclusion, the Third Canadian Workshop on Marine Harmful Algae was well attended and was very successful. The informal nature of the Workshop, and particularly, the manageable size of the six Working Groups, which were assigned specific topics and questions, proved to be effective, as acknowledged by many participants. The Fourth Workshop is planned for the Fall/Winter of 1993 at the Institute for Ocean Sciences in Sidney, British-Columbia. A recurrent suggestion was made by several members of the discussion groups to the effect that the next workshop should address more particularly the problem of standardization of methodology for cultivating toxic algae. It should also allow for a retrospective survey of historical phytoplankton data on the spatial distribution of *Dinophysis* spp. in Canadian waters.

ANNEX 1

OVERVIEW OF CANADIAN PHYTOPLANKTON MONITORING PROGRAMS

by

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INTRODUCTION

At the present time, approximately fifteen species of marine algae occurring in Canadian marine waters have the potential to cause harmful effects on both marine organisms and human consumers. This list continues to grow as scientific information increases. Many of these harmful algae can cause serious problems to the fishing industry, both wild and aquaculture. Three major groups of phycotoxins can affect shellfish: paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), and diarrhetic shellfish poisoning (DSP); and these are endemic on both east and west coasts. In addition, certain species of diatoms and chloromonads on the west coast have caused direct salmonid mortalities, but not thus far on the east coast.

To manage Canadian fisheries, it is essential to develop a sound understanding of the factors that influence the species composition of phytoplankton communities, especially the toxigenic species. Therefore, in 1987 the Department of Fisheries and Oceans (DFO) initiated a phytoplankton monitoring program on the Atlantic Coast.

This brief overview, written from a national perspective, describes the various projects that have been conducted, presents a few results, and outlines future plans. The focus is on the DFO's Science Sector program, but reference is also made to complementary programs in DFO's Inspection Services Branch, as well as industry.

DESIGN OF INITIAL PROGRAM

The first phytoplankton-monitoring project began in the Quoddy region of the Bay of Fundy in 1987, an area with a growing salmonid mariculture industry (Wildish et al. 1988). As a result of the domoic acid crisis in eastern Prince Edward Island (PEI) in late 1987, it was decided to establish an expanded monitoring program which covered the entire Atlantic Zone. A working group, with members from the Scotia Fundy, Gulf, and Québec Regions was convened in the spring of 1988 to develop objectives and protocols for sampling and analysis. It was agreed to undertake a 3-year program with the following objectives:

- Determine what areas and times are favourable or unfavourable for shellfish or finfish aquaculture with regards to the presence of toxins.
- Indicate times when screening for toxins should be more or less frequent if a consistent species succession can be established.
- The program would also provide background information for gauging whether observed phytoplankton events are normal or whether changes in biomass and

species diversity may be related to exceptional meteorological events or anthropogenic activity.

On the order of 24 coastal stations were initially selected, most of which were very near to existing mariculture facilities. Sampling frequency was monthly in the winter, fortnightly in the spring and fall, and weekly in the summer. It was recommended that variables measured should include: phytoplankton species enumeration, taxonomy and quantitative abundance, chlorophyll, temperature, salinity, and inorganic nutrients. Attention was also given to developing common database management methods. A summary of the program was presented at the First Canadian Workshop on Harmful Marine Algae (Gordon 1989). As is clear in the above objectives, this initial program was designed to improve scientific understanding of phytoplankton ecology, not to provide an operational early-warning system. In the Gulf Region an early-warning system for the presence of harmful algae was a component of their initial phycotoxin program.

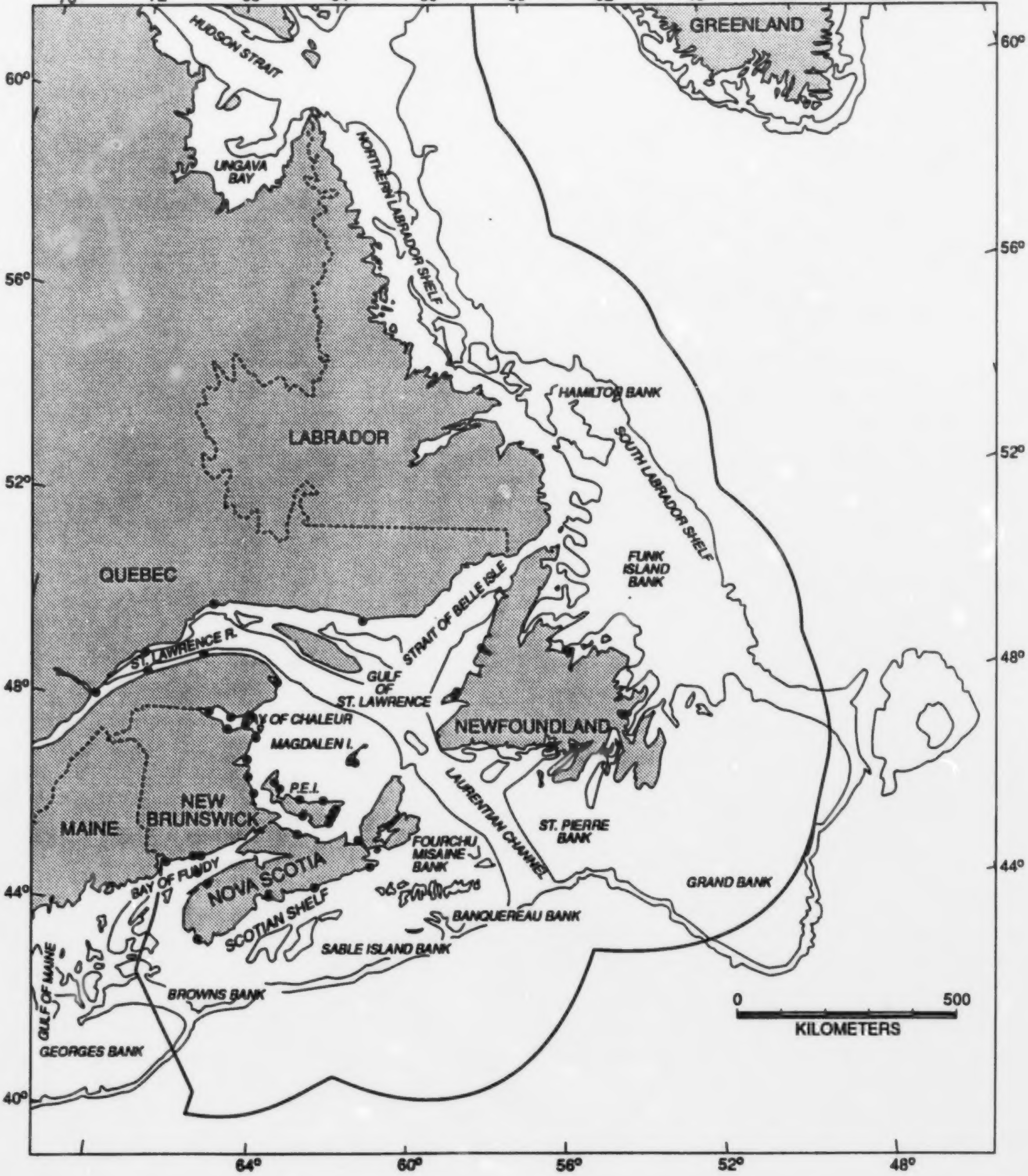
ACCOMPLISHMENTS OF THE ORIGINAL PROGRAM

Stations sampled as part of the core program are identified in Figure 1. Accomplishments vary somewhat by region, depending on the resources and expertise available. Some modifications were also made to program design as results were obtained and evaluated.

Québec Region - Phytoplankton identification and counts, temperature, and salinity data were collected over a 3-year period at ten stations. No nutrient data were collected. Results of 1989 sampling have been published (Larocque and Cembella 1991a; 1991b). Further reports will be published.

Gulf Region - Phytoplankton identification and counts, chlorophyll, temperature, salinity, and nutrient data were collected over a 4-year period at up to 40 stations in collaboration with Inspection Services Branch and others. In addition to this basic information, a number of other variables have been measured including irradiance and extinction, *in vivo* fluorescence, seston, particulate protein and amino acids, ^{15}N uptake rates, and ^{14}C photosynthesis. Offshore samples are obtained when opportunity permits (e.g. from Scotia-Fundy Region ice forecast cruises or from Gulf Region groundfish and herring surveys). Data reports and primary publications describing this work will be published in the near future.

Scotia-Fundy Region - Phytoplankton identification and counts, chlorophyll, temperature, salinity, and nutrient data were collected over a 4-year period at four stations by the St. Andrews Biological Station. Data collected through to 1989 have been published (Wildish et al. 1988; Wildish et al. 1989). Further reports will be



76°
72°
68°
64°
60°
56°
52°
48°
44°
40°
36°
GREENLAND
HUDSON STRAIT
UNGAVA BAY
NORTHERN LABRADOR SHELF
HAMILTON BANK
SOUTH LABRADOR SHELF
LABRADOR
QUEBEC
FUNK ISLAND BANK
STRAIT OF BELLE ISLE
ST. LAWRENCE R.
GULF OF ST. LAWRENCE
BAY OF CHALEUR
MAGDALEN I.
P.E.I.
NEWFOUNDLAND
LAURENTIAN CHANNEL
ST. PIERRE BANK
NEW BRUNSWICK
MAINE
FOURCHU MISAINE BANK
BAY OF FUNDY
NOVA SCOTIA
SCOTIAN SHELF
BANQUEREAU BANK
SABLE ISLAND BANK
GRAND BANK
GULF OF MAINE
BROWNS BANK
GEORGES BANK
0
500
KILOMETERS
60°
56°
52°
48°
44°
40°

Figure 1. Stations sampled as part of the core phytoplankton monitoring program.

published. Taxonomy, cell counts, chlorophyll, nutrient, suspended particulate matter and light data, as well as profiles of in situ fluorescence, temperature and salinity, were collected over a 3-year period at five stations by the Bedford Institute of Oceanography (BIO). Photocopied interim reports (Keizer 1991a; 1991b; 1991c; 1991d; 1991e) have been prepared as well as a summary for the "Science Review" (Bugden et al. in press). A technical report should be available in 1992 covering the 3-year study.

Newfoundland Region - Taxonomy, cell counts, temperature, and salinity were collected over a 3-year period (1989-92) at two stations: one at Charles Arm in Notre Dame Bay on the northeastern coast of the island, and the second at Pools Cove in Fortune Bay on the island's southern coast. Suspended particulate matter data are also collected at the Charles Arm site. Both sites are active shellfish farms. Data collected in 1989 have been included in two reports (McKenzie et al. 1990a; 1990b). Further reports and publications are pending for the 1990-92 data.

BRIEF SUMMARY OF THE RESULTS OBTAINED IN THE ATLANTIC ZONE PROGRAM

Phytoplankton Taxonomy - Species which have been shown to produce phycotoxins under certain conditions or to damage the gills of salmonids are listed in Table 1. ASP-producing species include *Nitzschia pungens* f. *multiseries*, *N. delicatissima*, and *N. pseudodelicatissima*; PSP-producing species include *Alexandrium funyense*, *A. excavatum*, and *A. tamarense*; DSP-producing species include *Dinophysis norvegica*, other *Dinophysis* species, and *Prorocentrum lima*. Species which are known to be harmful to finfish include *Gyrodinium aureolum* and *Chaetoceros concavicornis*, which is capable of damaging the gills of salmonids. While usually present in low numbers, it is clear that potentially toxigenic species are found at each station and that most are widespread in the Atlantic region. Therefore, there is the potential that toxic events could occur almost anywhere if the environmental conditions are right.

Physical Oceanography - The five stations sampled by BIO represent a wide variety of conditions with respect to their geography, tidal range, freshwater run-off, and aquaculture activity. For example, Woods Harbour and Annapolis Basin have the largest ratios of tidal to non-tidal volume, suggesting that tidal exchange is important at these sites. Annapolis Basin, Ship Harbour, and Tor Bay have comparable, relatively large, ratios of freshwater discharge to non-tidal volume, suggesting the potential importance of this driving force. The annual range of temperature was greater at Tor Bay, Ship Harbour, and St. Margaret's Bay resulting in winter ice formation. Summer temperatures at these three inlets were also higher than at Woods Harbour and Annapolis Basin. This suggests that the larger tidal exchange with the ocean moderates the temperature throughout the year at these two sites. At the stations sampled by BIO, increased vertical resolution of physical variables was achieved

Table 1. Listing of potentially toxic or harmful phytoplankton species which were detected at the monitoring stations. Most species were found in very low numbers.

Station Number	Location	Potentially Toxic/Harmful Species
1	Sainte-Flavie	*Alexandrium* spp., *Dinophysis* spp.
2	Baie-Comeau	*Alexandrium* spp., *Dinophysis* spp.
3	Gaspé	*Alexandrium* spp., *Dinophysis* spp.
4	Gascons	*Alexandrium* spp., *Diniphysis* spp.
5	Sept-Iles	*Alexandrium* spp., *Dinophysis* spp.
6	Port-Daniel	*Alexandrium* spp., *Dinophysis* spp.
7	Carleton	*Alexandrium* spp., *Dinophysis* spp.
8	Grande-Entrée	*Alexandrium* spp., *Dinophysis* spp.
9	Baie-des-Capucins	*Alexandrium* spp., *Dinophysis* spp.
10	Tadoussac	*Alexandrium* spp., *Dinophysis* spp.
11	SE Gulf St. Lawrence	*Alexandrium excavatum*, *Nitzschia pungens* f. *multiseries*, *N. delicatissima*, *Prorocentrum* spp., *Chaetoceros concavicornis*, *Dictyocha speculum*, *Gyrodinium* spp.
12	Lime Kiln Bay	*Alexandrium fundyense*, *Nitschia pseudodelicatissma*
13	Deadman Harbour	
14	Brandy Cove	
15	The Wolves	
16	Digby	*Alexandrium* spp., *Dinophysis* spp., *Prorocentrum* spp., *Nitzschia pungens* f. *multiseries*, *Nitzschia pseudodelicatissima*
17	Woods Harbour	*Alexandrium* spp., *Dinophysis* spp., *Prorocentrum* spp., *Nitzschia pungens* f. *multiseries*
18	St. Margaret's Bay	*Alexandrium* spp., *Dinophysis* spp., *Prorocentrum* spp., *Nitzschia pungens* f. *multiseries*, *Nitzschia pseudodelicatissma*, *Chaetoceros concavicornis*
19	Ship Harbour	*Dinophysis* spp., *Prorocentrum* spp., *Nitzschia pungens* f. *multiseries*, *Nitzschia pseudodelicatissma*
20	Tor Bay	*Dinophysis* spp., *Prorocentrum* spp., *Nitzschia pungens* f. *multiseries*, *Nitzschia pseudodelicatissma*
21	Notre Dame Bay	
22	Fortune Bay	

through the use of a portable CTD. Increased temporal resolution of temperature, which may be used to indicate exchange with offshore waters, was obtained from thermographs moored at each sampling site. This enhanced physical data set is being used to characterize interaction with offshore waters at each site. In the Québec Region, results obtained at ten stations sampled by the Maurice Lamontagne Institute (MLI) during the monitoring program (3 years of data) support the hypothesis that the distribution of *Alexandrium* sp. is largely confined to the plumes of the Manicouagan and Aux-Outardes Rivers in the lower St. Lawrence Estuary and to the Gaspé Current in the Gulf.

Chemical Oceanography - The nutrient distributions at the five sites sampled by BIO varied in a manner that is typical of temperate coastal waters. Concentrations of silicate, nitrate, and phosphate were high during the winter months with substantial and rapid reductions in concentrations occurring in the spring as the phytoplankton populations increased. The initial spring depletion of nutrients was generally followed by increased concentrations in the late spring/early summer, and then very low levels were observed for periods of variable duration. In the late fall, a return to the elevated wintertime concentrations was seen. The observed pattern was similar for silicate, nitrate, and phosphate, but essentially complete removal occurred only for nitrate. The nutrient concentrations were generally uniform from surface to bottom at these rather shallow sampling sites. The exception was Ship Harbour where marked vertical gradients and extremely high concentrations in the bottom waters were found for phosphate, silicate, and ammonia in summer and fall.

Database Management - In the initial stages of the program, several meetings were held to discuss database management and to agree on common approaches wherever possible. The St. Andrews Biological Station developed a master list of phytoplankton species with code numbers to which other regions have contributed. The Québec Region developed a data-entry program which they made available to other Regions. Each Region subsequently developed their own database and assumed the responsibility for maintaining it. BIO has put its data into a FoxPro database management system and is pleased with the ease of access. No attempts have yet been made to exchange data between Regions; but, because of the initial coordination steps taken and the compatibility of new software, this should be a relatively straightforward task.

Publications - A number of publications based on data collected have been released and are listed in the "References" section in this report. These are mostly in the form of interim reports, abstracts, and technical reports.

Pacific Region - In British Columbia coastal waters, there are 13 confirmed and one probable harmful/toxic species present (Table 2).

Table 2. Toxic and harmful species in British Columbia waters.

Confirmed Present	Effect
Dinophysis acuminata	DSP
Alexandrium tamarensi	PSP
Alexandrium catenella	PSP
Alexandrium acatenell	PSP
Cochlodinium citron	PSP?
Nitzschia pungens f. *multiseries*	ASP
Nitzschia pseudodelicatissima	ASP
Heterosigma akashiwo	Fish kill
Undescribed *Chloromonad* species	Fish kill
Chaetoceros convolutum	Fish kill
Chaetoceros concavicorne	Fish kill
Gymnodinium flavum	Fish kill
Prorocentrum minimum	Liver damage (in clams and oysters)

ESTABLISHMENT OF PHYTOPLANKTON WATCH PROGRAMS

As described above, the initial 3-year program had rather broad objectives to improve our scientific understanding of the occurrence of harmful phytoplankton species. It did not address the needs of DFO Inspection Services Branch and the aquaculture industry to have an early warning of blooms of potentially toxic species. Therefore, a number of phytoplankton watch projects have been established which involve DFO Science, DFO Inspection Services Branch, provincial agencies, and industry. These are reviewed below, by Region. These projects focus on those few species which are potentially dangerous and provide information on the day of sampling.

Pacific Region - A phytoplankton watch project was established in the summer of 1986 after a massive bloom of *Heterosigma akashiwo*, which caused heavy fish mortality. It has been operated and funded jointly by the aquaculture industry and the provincial government. The project focuses on education, data gathering, communication, and standards. Until this year, a project coordinator has, among other duties, alerted fish farms of impending harmful bloom situations. A toll-free 1-800 telephone line and direct telephone calls to each farm were the main communication links. The project is being reorganized this year, being decentralized to areas where fish farms are congregated, such as northern Vancouver Island and the west coast of Vancouver Island. The new structure has not yet been finalized.

Québec Region - DFO Science immediately screens samples collected as part of its regular program for potentially dangerous species and reports any evidence of developing blooms to DFO Inspection Services Branch.

Gulf Region - Starting in 1988, DFO Science first operated and then helped the Inspection Services Branch set up its own phytoplankton watch project. Samples are collected on a regular basis at over 30 locations. Phytoplankton monitoring is also conducted by the PEI Department of Fisheries and Aquaculture at 16 sites throughout the province (Bernard 1991).

Scotia-Fundy Region - The St. Andrews Biological Station immediately screens samples collected as part of its regular program for potentially dangerous species and reports any evidence of developing blooms to DFO Inspection Services Branch in Blacks Harbour. Inspection Services Branch in Halifax has recently initiated a phytoplankton watch project along the Atlantic coast of Nova Scotia which is conducted under contract through the Aquaculture Association of Nova Scotia using Economic Regional Development Agreement (ERDA) funding arranged by the Nova Scotia Department of Fisheries (up to October 1992).

To improve the exchange of information on phycotoxin events, a bloom alert network has been established in the Atlantic Zone. Members include DFO Science, Inspection Services Branch, National Research Council, provincial agencies, and the aquaculture industry. Members transmit information of interest by way of fax.

PLANS FOR THE FUTURE

An inventory of all available Canadian databases, including phytoplankton taxonomy, is being compiled by R. Forbes of the Institute of Ocean Sciences. A progress report has been presented at the Mont-Joli workshop.

After 5 years of experience in monitoring phytoplankton, DFO has identified four different kinds of phytoplankton-monitoring projects which are defined as follows.

Long-Term Trend Monitoring - The objective of long-term trend monitoring is to seek and explain gradual changes in species composition and abundance over decadal time scales. They require a long-term commitment at the outset. These stations should be limited in number, selected with care, and institutionalized so that their continuance is not dependent on specific individuals.

Monitoring in Support of Research Programs - These projects will be somewhat flexible and change in design with time depending on results. The initial 3-year DFO Science program falls under this category.

Samples Opportunity - Despite the numerous projects undertaken in recent years, there are many areas important to fisheries for which we have very little information on phytoplankton taxonomy. For example, all stations sampled to date are coastal, and there are none on offshore fishing banks. It is, therefore, important to collect samples on an opportunistic basis whenever conditions allow, even if only once each year.

Phytoplankton Watch Monitoring - As defined above, this type of monitoring provides an early warning of potential problems to regulatory agencies and industry. This can also be a valuable source of biogeographic and floristic data.

Regional plans for continuing phytoplankton monitoring projects are reviewed below.

Pacific Region - DFO is initiating a research project at three to four sites in the Strait of Georgia and on the west coast of Vancouver Island. The major objective is to improve our understanding of the physical and chemical processes leading to monospecific blooms of harmful species in the Region. The nature of the sampling program will also allow the data to be used for monitoring purposes. The sites are chosen so that they may later be incorporated into a long-term trend monitoring project. In addition, sampling kits have been placed on DFO patrol and science vessels, which, with coordinated reports from Fisheries Branch aerial patrols, allows for comprehensive monitoring of opportunity along the coast (Forbes 1991). The industry-supported phytoplankton watch program will continue in a new format, including links to a companion program in Washington State (Horner et al. 1991).

Central and Arctic Region - Tentative Regional plans for phytoplankton monitoring for the coming year will include: 1) Monitoring the phytoplankton, both pelagic and benthic if possible, from the mussel sampling sites on the Yellowknife and Cameron Rivers in the Northwest Territories. It is hoped to be able to get phytoplankton samples to Winnipeg every 3 weeks through the open-water season and enumerate species and biomass. Culturing and isolation of specific species will be attempted if and when a bloom appears to be developing. 2) Monitor acidified lake-302 South at ELA for blooms of the dinoflagellates *Gymnodinium* and *Peridinium*, concentrate by selective filtration, and analyze for toxic compounds. Also attempt to get one or both of these algae into culture. Neither of the above are long-term projects and will be assessed at the end of the year to see if they are worth continuing given the fact that costs will have to be covered by other projects. Not being planned for the coming year is any further research on the east coast. Sufficient data have been gathered, and analysis is underway.

Québec Region - The current 10 stations will continue. A long-term trend-monitoring station will be established at Sainte-Flavie (MLI). Nutrient concentrations (nitrate, nitrite, silicate, phosphate, ammonium, and urea) will be determined weekly at this station. An additional two to three stations will be sampled in the Magdalen Islands and in the Baie-des-Chaleurs in support of research projects. DFO Science will continue to screen samples and pass along to Inspection Services Branch any information on pending blooms.

Gulf Region - The establishment and location of long-term trend monitoring stations are being considered. Monitoring in support of research projects will continue at New London Bay, Cardigan, Brudenell, Murray, and Miramichi Rivers. DFO Inspection Services Branch will continue to operate the phytoplankton watch project at about 30 stations.

Scotia-Fundy Region - Four long-term trend monitoring stations are being established. Three of these stations were established as part of the initial DFO program (Lime Kiln Bay, The Wolves, and St. Margaret's Bay). The fourth will be established at Sambro Head at the mouth of Halifax Harbour near Chebucto Head. Monitoring at Annapolis Basin and Ship Harbour will continue, although with altered sampling schedules, for at least 1 year in support of ongoing process-oriented studies. Monitoring will also be done on an opportunistic basis on Georges Bank, Western Bank, and in the Bras d'Or Lakes. St. Andrews will continue to screen samples collected in the Quoddy region for problem species and alert Inspection Services Branch if necessary. The Aquaculture Association of Nova Scotia will continue to conduct the phytoplankton watch project until at least October 1992 (when current funding expires).

Newfoundland Region - The phytoplankton project at two shellfish farms was completed as of March 1992. No further plans for phytoplankton monitoring have been finalized as yet.

It is very important that the data collected in all phytoplankton monitoring projects are properly managed, analyzed, reported, and made available to interested parties. As discussed above, each Region has the responsibility of constructing and maintaining its own database. A good start has already been made with data analysis, and several technical publications have appeared. It is anticipated that scientific papers will soon be forthcoming. At some stage in the near future, it would be interesting to compare the data from different regions and prepare a zonal overview. The total database provides a wealth of information for comparing the environmental properties and dynamics of coastal inlets, estuaries, and bays - especially in the Atlantic region.

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ANNEX 2

REVIEW OF
THE PHYCOTOXINS RESEARCH PROGRAM IN THE DEPARTMENT OF FISHERIES AND OCEANS (DFO)

Prepared by the DFO Phycotoxins Working Group

April 1992

EXECUTIVE SUMMARY

The Science Sector of the Canadian Department of Fisheries and Oceans (DFO) conducts a subtantiel national research program on harmful marine algae. Thirty-three projects are currently being conducted out of laboratories based in St. John's, Nfld., Halifax/Dartmouth, N.S., St. Andrews, N.B., Moncton, N.B., Mont-Joli, P.Q., Winnipeg, Man., Sidney, B.C., and Naniamo, B.C., under the general categories of: 1) methodology and analytical support, 2) phytoplankton population dynamics, 3) biological and biochemical aspects of toxin production, 4) uptake, storage and depuration of toxins by marine organisms, 5) effects of toxins on marine organisms, 6) fate of toxins, and 7) physical oceanography, chemical oceanography, and sedimentology. Many projets are carried out in collaboration with DFO Inspection Services Branch, the National Research Council, the Department of Health and Welfare, universities, provincial departments that deal with fisheries and aquaculture, and industry. In addition, DFO scientists organize national workshops and participate in international working groups and committees dealing with harmful marine algal issues. In collaboration with regulatory agencies, research results are applied to the management of harmful marine algal episodes to protect consumers of seafood as well as the fishing industry (wild and aquaculture).

INTRODUCTION

Harmful marine algae are widely distributed on both the Pacific and Atlantic coasts of Canada. Three major groups of well-defined toxins, of concern to human consumers of shellfish - paralytic shellfish poisons (PSP), amnesic shellfish poison (ASP), and diarrhetic shellfish poisons (DSP) - are produced by a limited number of marine algal (phytoplankton) species. Some phytoplankton species, such as *Heterosigma akashiwo*, cause toxic reactions in fish that are poorly understood. Other species, such as *Chaetoceros concavicornis*, can cause harmful effects by damaging the gills of fish.

Prior to 1988, DFO conducted only a limited research program on harmful marine algae. Following the ASP (domoic acid) outbreak in Prince Edward Island (PEI) mussels, the Science Sector of DFO expanded is program in order to help solve the problems created by harmful marine algae.

In 1989, DFO created a national in-house advisory group called the Phycotoxins Working Group (PWG) which is made up of representatives from all DFO Regions. The PWG reports to the Biological Sciences Subcommittee composed of Biological Sciences directors from all DFO Regions.

The present review of the national DFO phycotoxins program has been prepared by the PWG with the intent to inform research scientists, managers, industry and the interested public. It contains: 1) a table summarizing all current DFO phycotoxins research projects across the country, 2) highlights of recent research, 3) general observations and recommendations from the PWG, 4) a list of hypotheses developed by the PWG to help guide future research, and 5) a list of publications on phycotoxins research since 1986.

LISTING OF CURRENT PROJECTS

This list shows current DFO research projects on harmful marine algae from all Regions, categorized by topic. Projects are assigned to one of the seven categories, but are cross-referenced to other categories where appropriate. The DFO Regions are abbreviated as follows: GUL = Gulf Region, S/F = Scotia-Fundy Region, QUE = Québec Region, C&A = Central and Arctic Region, and PAC = Pacific Region.

REGION	PROJECT TITLE (SCIENTIFIC LEADER)	TOXINS/SPECIES	CROSS-REFERENCE
1. METHODOLOGY AND ANALYTICAL SUPPORT			
S/F	Techniques and improvements (Zitko)	ASP, DSP	
S/F	Investigations into shellfish toxins (Pocklington)	ASP, DSP, PSP	2,3,4,6
2. PHYTOPLANKTON POPULATION DYNAMICS			
PAC	Toxic algal blooms (Forbes)	All species	1,7
C&A	Phytoplankton nutrient status (Guildford)	All species	
QUE	Programme de suivie des populations d'algues nuisibles dans l'estuaire et le nord du golfe Saint-Laurent (Levasseur)	PSP, DSP	
QUE	Introduction d'algues toxiques par les eaux et sédiments de ballast aux Iles-de-la-Madeleine (Golfe du Saint-Laurent) (Gosselin)	PSP, DSP	
QUE	Dynamique de population des algues toxiques et nuisibles dans le Saint-Laurent: 1) Germination des kystes d'*Alexandrium excavatum* (Levasseur)	PSP	
GUL	Physiological ecology of harmful and benign phytoplankton (Smith)	All species	1,2,4,5,6,7
GUL	Nutrient dynamics and phycotoxin production in the field and laboratory (Cormier, Smith)	All species	1,3,7
S/F	Coastal phytoplankton dynamics (Keizer)	All species	
S/F	Phycotoxin bloom dynamics in the Fundy Isles area (Martin)	All species	2,7
S/F	Physiological ecology of toxic algae (Subba Rao)	ASP, DSP	3,4
S/F	Ballast waters as a source of algal blooms (Subba Rao)	All species	

Region	Project title (Scientific leader)	Toxins/species	Cross-reference
3. BIOLOGICAL AND BIOCHEMICAL ASPECTS OF TOXIN PRODUCTION			
PAC	Toxic algae (Whyte)	ASP, *Heterosigma*	1,2,4,5
QUE	Growth and physiological studies of *Alexandrium* spp. (Hsiao)	PSP	
GUL	Biology of toxin producing phytoplankton (Bates)	ASP	2
S/F	Factors controlling the production of domoic acid in the Bay of Fundy (Martin)	ASP	2
4. UPTAKE, STORAGE, AND DEPURATION OF TOXINS BY MARINE ORGANISMS			
C&A	Investigation of freshwater mussels from the Northwest Territories for unknown, low level toxicity (Hendzel)		
QUE	Approche flux cytométrique de l'etude de la sélectivité du broutage de la moule bleu (*Mytilus edulis*) (Demers)	PSP	
GUL	Effects of phycotoxins and other stressors on the condition, production, and marketability of molluscs (Smith)	All species	1,2,3,5,6
S/F	Molluscan culture and phycotoxin research (Scarratt)	ASP	
5. EFFECTS OF TOXINS ON MARINE ORGANISMS			
QUE	Hydrodynamisme, distribution des phytotoxines et effets sur le réseau alimentaire et la survie d'espèces commerciales (Gagné)	PSP	
QUE	Feeding responses and toxin accumulation by zooplankton grazing on toxic dinoflagellates (Runge)	PSP	
S/F	Effects of toxic microalgae on finfish (Wildish)	All species	
S/F	Aquatic toxicology and marine phycotoxins (Haya)	ASP, PSP	2,3,4
6. FATE OF TOXINS			
S/F	Microbial-marine toxin interactions (Stewart)	ASP, PSP	1,3
7. PHYSICAL OCEANOGRAPHY, CHEMICAL OCEANOGRAPHY, AND SEDIMENTOLOGY			
PAC	Red tide prediction (Murty and Gower)	All species	2
S/F	Long-term temperature monitoring (Petrie)	All species	2
S/F	Exchange between coastal and offshore waters (Bugden)	All species	2
S/F	Physical oceanography in support of phytoplankton profiling program (Bugden)	All species	2
S/F	Classification of estuaries, inlets and coastal embayments (Petrie)	All species	2
S/F	Suspended particulate matter associated with phytoplankton variability (Kranck)	All species	2
S/F	Nutrient dynamics in Ship Harbour (Strain)	All species	2

OVERVIEW

While there has been a long history of phycotoxins research in Canada, an expanded DFO phycotoxins program began in 1988 as a result of the domoic acid crisis in PEI in late 1987. New resources were allocated by Treasury Board for expanded research in the Québec, Gulf, and Scotia-Fundy Regions. New phycotoxin problems are occurring in other Regions as well and new research projects are being initiated, but without new resources.

Successful delivery of the DFO phycotoxins research program is very much associated with an effective collaboration with other agencies, especially DFO Inspection Services Branch, the Institute of Marine Biosciences (IMB) of the National Research Council, the Department of Health and Welfare, universities, provincial departments which deal with fisheries and aquaculture, and industry. Communication among the parties has been good. Each year, DFO scientists participate in a variety of meetings and workshops which deal with the planning, execution, and evaluation of phycotoxins research.

During the past year, DFO organized two workshops relevant to phycotoxins. A 1-day national workshop on the Risk to Canada's Marine Resources of Species Introductions Carried in Ships' Ballast Water was held at the Bedford Institute of Oceanography (BIO) on April 24, 1991. A 1-day workshop on Pacific Coast Research on Toxic Marine Algae was held at the Institute of Ocean Sciences (IOS, in Sidney, B.C.) on April 30, 1991. The Third Canadian Workshop on Harmful Marine Algae, being organized by DFO, was held at the Maurice Lamontagne Institute (MLI, Mont-Joli, Qué.) on May 12 to 14, 1992.

DFO scientists play prominent roles in international conferences dealing with phycotoxins and are members of several international committees, including the ICES Working Group on Phytoplankton and the Management of their Effects, the ICES Study Group on the Dynamics of Harmful Algal Blooms, the SCOR Working Group on the Physiological Ecology of Harmful Algal Blooms, and the IOC-FAO ad hoc Intergovernmental Panel on Harmful Algal Blooms. Some participated in the IOC-SCOR workshop on program development for harmful algal blooms. DFO researchers attended American workshops and have been advising United States authorities during the recent domoic acid event on the Pacific coast.

RESEARCH HIGHLIGHTS

Pacific Region

- General

A workshop on Pacific coast research on harmful marine algae was held at the Institute of Ocean Science (Sidney, B.C.) on April 30, 1991, and was attended by 60 scientists from DFO, the provincial government, industry, universities, and the United States. The proceedings have been published (Forbes 1991).

In late 1991, domoic acid was reported in shellfish from California, Oregon, Washington, and Alaska. Domoic acid was not detected in British Columbia in adjacent waters. Research programs are being planned to understand processes that may lead to its appearance in British Columbia.

- Methodology and Analytical Support

Experimental work continues with a spectroradiometer and spectrofluorometer, in combination with HPLC pigment analysis, to improve methodology for rapid characterization of phytoplankton community structure in the field. (Forbes)

- Phytoplankton Population Dynamics

Data on phytoplankton identity and abundance from British Columbia coastal waters collected from 1979 to 1989 have been organized for publication as a data report. Interpretation of the data in terms of spatial and temporal variability is in progress. (Forbes)

Sampling kits have been distributed to Fisheries Patrol and Science vessels to obtain taxonomic samples of major algal blooms when they occur along the coast. In conjunction with reports from Fisheries Branch aerial patrols and the aquaculture industry, this should provide comprehensive documentation of the frequency, location, and type of blooms. Underway fluorescence systems are maintained on Science vessels to establish seasonal variability of phytoplankton biomass on the continental shelf. (Forbes)

A new project will initiate long-term sampling of phytoplankton species composition and water properties at a small number (three to five) of sites selected for dissimilar oceanographic conditions. (Forbes)

- Biological and Biochemical Aspects of Toxin Production

Field and laboratory bioassays with juvenile salmonids have demonstrated that fish mortality from exposure to *Heterosigma akashiwo* is caused by a toxin as yet not isolated or identified. Work will continue on identification of the toxin and on establishing the physiological mechanisms for toxin production. (Whyte)

A new project will investigate the conditions of toxin production in algae, the incorporation and physiological effects of toxins on marine species, the nature of the toxins and the mechanisms for detoxification of commercial species. This project will concentrate initially on domoic acid. (Whyte)

- Physical and Chemical Oceanography

An evaluation of the sensitivity of the AVHRR satellite sensor to extreme concentrations of phytoplankton in coastal waters was completed. Minimum cell concentrations required to establish a signal were calculated, and sea surface warming caused by absorption of solar irradiance by phytoplankton cells was measured. (Gower)

A new project is underway which will attempt to use numerical modelling of circulation in the Strait of Georgia, combined with phytoplankton growth parameters, to predict the conditions suitable for bloom formation. Satellite images and surface measurements will be used to check and fine tune the model. (Murty and Gower)

Central and Arctic Region

- General

This Region had no formal phycotoxins projects until FY 1992/93.

In November 1991, the presence of low-level, PSP-like toxicity was reported by the Inspection Services Branch for freshwater mussels from two locations in the Northwest Territories. Subsequent follow-up analyses failed to show any further toxicity in either mussel tissue or phytoplankton samples.

- Phytoplankton Population Dynamics

In cooperation with the Gulf Region, nutrient status and photosynthesis were measured during November in coastal areas of PEI. (Guildford)

- Uptake, Storage, and Depuration of Toxins by Marine Organisms

A new project is being designed to investigate the mussel toxicity reported in 1991 in the Northwest Territories. (Hendzel)

Québec Region

- General

The phycotoxins program in the Québec Region has made adjustments as a result of the departure of A. Cembella. M. Levasseur has taken over project leadership.

- Phytoplankton Population Dynamics

The role of ballast water as a source of toxic algal species in the Magdalen Islands continues to be evaluated. The information obtained from this project will help assess the magnitude of the problem of transport of harmful algae in ballast water and will contribute to evaluation of the risk to the aquatic ecosystem. In addition, it should be possible to verify the efficacy of ballast water exchange as a preventative measure. (Gosselin)

Work continues on understanding the endogenous rhythm in the germination of *Alexandrium excavatum* cysts. It has been proposed that this process may be more important than environmental factors in causing blooms. Increased understanding should improve the capacity to predict bloom events. (Levasseur)

A technical report on the monitoring program for 1989/1990 has been completed and published (Larocque and Cembella 1991). All of the net tow samples from 1990/1991 have been analyzed, as well as the associated physical/chemical information, and data have been transferred to a database. A technical report will be produced on the 1990/1991 monitoring program. (Levasseur)

- Effects of Toxins on Marine Organisms

Work continues on assessing the effect of neurotoxins of *Alexandrium excavatum* on fish larvae. In the laboratory, fish larvae and early post-larvae proved highly vulnerable to the neurotoxins of *A. excavatum*. However, a preliminary analysis for Atlantic herring in the southern Gulf of St. Lawrence does not support the hypothesis that dinoflagellate toxicity has an impact on recruitment, but this could very well results from the inadequacy of the data set. (Gagné)

Work also continues on assessing the effect of neurotoxins on copepods. Feeding experiments on *Calinus finmarchicus* show that when fed on *A. excavatum*, it ceases to feed on the very toxic strains in less than 2 hours, but that longer-term ingestion of toxic algae can take place in the presence of other palatable food cells. The degree of feeding and toxin accumulation therefore appears to depend on the toxin level in individual algal cells and the relative proportion of toxic algae to total food concentration in the water. (Runge)

Gulf Region

- General

Despite the progress made in recent years, our understanding of harmful algal blooms is still inadequate and prediction of toxic events is difficult. Nevertheless, the management of many phycotoxin problems is under control. For example, the phytoplankton and shellfish monitoring program run by the DFO Inspection Services Branch was able to detect the development of an unexpected toxic bloom of *Nitzschia pungens* f. *multiseries* in New London Bay, PEI, and appropriate regulatory procedures were taken to protect consumers.

- Methodological and Analytical Support

A set of methods designed to ensure effective monitoring of toxic phytoplankton has been assembled and field tested. Training in these techniques has been provided to a large number of clients, including Inspection Services Branch in the Gulf and Scotia-Fundy Regions, the National Research Council and university personnel. The methods are embodied in a manual which has been thoroughly tested in the field and which will be published in the near future. (Smith)

- Phytoplankton Population Dynamics

Field studies have indicated that the supply of combined inorganic nitrogen is related in a complex fashion to both the species succession preceding a toxic bloom of *Nitzschia pungens* f. *multiseries* and to the biomass and toxicity achieved during the bloom. Also, *N. pungens* f. *multiseries* grows faster and outcompetes both *N. pungens* f. *pungens* and other similar phytoplankton with which it co-occurs in the late fall. (Smith)

Studies of the biogeography, floristic composition, species succession, production, and dynamics of phytoplankton populations over large spatial and temporal scales have shown that a broad range of toxic algae and related species exists in the Gulf Region.

They have also provided information on where and when various types of toxic events might be anticipated. It is evident that there is large interannual variability in the timing and location of toxic algal blooms. (Smith)

In the case of *N. pungens* f. *multiseries*, it appears that several factors must be favourable for a bloom to occur and that some of these factors operate over a time scale of months. These factors include: a) a long period of dry weather in the summer with retention of terrestrial nutrients on land, b) a period of nutrient limitation in the water in the early fall during which *N. pungens f. multiseries* successfully competes for nutrients and largely replaces other phytoplankton, c) a period of heavy rain which flushes large amounts of nutrients into the system in a brief period, and, following this pulse of nutrients, d) a period of fairly calm weather such that the growing algal population is not advected away. (Smith)

- Biological and Biochemical Aspects of Toxin Production

Factors controlling the growth of the diatom *Nitzschia pungens* f. *multiseries* and its production of domoic acid are being elucidated using laboratory cultures. Domoic acid is produced only after the diatom cells stop dividing due to silicate depletion during the stationary phase in batch culture. Nitrogen and light are required for domoic acid production. High, possibly toxic, concentrations of ammonium enhance domoic acid production, as do Tris buffer and calcium carbonate when added to the culture medium. No unique physiological adaptations were found to the low temperatures characteristic of the diatom's natural growth environment in the autumn, but cell division saturates at an unusually low irradiance level. Less domoic acid is produced when irradiance decreases below a threshold level. Several clones of *N. pungens* were made bacteria free (axenic) using antibiotic treatment. Axenic clones produce domoic acid, but at a level 20 times less than bacteria-containing cultures. Experiments are continuing to determine if the antibiotic treatment affected the cells' physiology. An immunofluorescence technique is being developed to distinguish the domoic acid-producing form (f. *multiseries*) from the non-toxic form (f. *pungens*) of *N. pungens* by raising antibodies against cell surface proteins of whole cells. (Bates)

During this year we have successfully brought into culture for toxicity testing approximately 50 clones of each of *N. pungens* f. *multiseries* and *N. pungens* f. *pungens* as well as a number of clones of *Prorocentrum lima*, *P. minimum*, and various other species of *Prorocentrum*, together with other pennate diatoms of interest. (Smith)

Scotia-Fundy Region

- General

A workshop to assess the risk to Canada's marine resources of species introductions carried in ships' ballast water was held at the Bedford Institute of Oceanography on April 24, 1991. The proceedings were published as a technical report (Smith and Kerr 1992).

- Methodology and Analytical Support

An alternative method of chromatography for PSP toxins, involving pre-column oxidation, was successfully implemented and a new derivatization procedure for the determination of DSPs by HPLC-fluorescence is under evaluation. A major impediment to analytical advances remains the absence of adequate standard materials for the calibration of PSP and DSP analyses, a problem being dealt with by the Institute for Marine Biosciences of the National Research Council. Domoic acid determinations continue to be carried out for a variety of clients within and outside of DFO. (Pocklington)

Analytical support is provided to Regional phycotoxins projects including measurements of domoic acid by HPLC and okadaic acid by the UBE kit. (Zitko)

A tissue culture bioassay method for the detection of PSP has been modified to increase its speed and convenience. It was compared with the mouse bioassay in a collaborative study with Inspection Services Branch. The results obtained by the two methods were essentially identical. The tissue culture method, however, is more sensitive, faster, and does not require the use of live animals. With this method, any laboratory with basic microbiological equipment can perform their own PSP analyses. This method has considerable potential for regulatory agencies and research laboratories. (Stewart)

- Phytoplankton Population Dynamics

A study of the phytoplankton population dynamics in the southwestern Bay of Fundy was initiated in 1987. *Alexandrium fundyense*, *Nitzschia pseudodelicatissima*, and *Dinophysis* sp., which produce the toxins causing PSP, ASP, and DSP respectively, have been observed in significant concentrations. *Chaetoceros convolutus*, *Gyrodinium aureolum*, and *Nitzschia pungens*, organisms that have been implicated in harmful events elsewhere, have also been observed. Preliminary analysis indicates that there are no obvious correlations among phytoplankton abundance and temperature, salinity, and concentrations of chlorophyll "a," silicate, phosphate, and nitrate. (Martin)

A 3-year phytoplankton monitoring program, which included five coastal sampling sites along the Fundy and Atlantic coasts of Nova Scotia, was completed. Interim reports were prepared and distributed to interested parties, including the aquaculture industry. Potentially harmful phytoplankton species identified include *Alexandrium* sp., *Dinophysis* sp., *Prorocentrum* sp,. *Nitzschia pungens*, and *Nitzschia pseudodelicatissima*. Physical, chemical, and biological data have been entered into a FoxPro database management system. (Keizer)

Several algae, new to Canadian coastal waters, were isolated from ballast water samples supplied by G. Sprules (University of Toronto) and are being cultured. Analysis of more than 50 preserved water samples revealed the presence of phytoplankton taxa new to Canadian coastal waters, including a few potentially toxigenic species. Under the right conditions, some of these algae could develop episodic blooms. (Subba Rao)

- Biological and Biochemical Aspects of Toxin Production

Domoic acid was detected in phytoplankton tows collected during 1990/1991 in Passamaquoddy Bay. The highest concentration observed was 3.9 µg/g wet weight (September 1990). Unialgal cultures of *Nitzschia pseudodelicatissima* have been isolated from water collected in the same area. Domoic acid has been detected in the log phase and lag phase of growth. Concentrations of domoic acid from 7.0×10^{-16} to $9.8\ x^{-14}$ g/cell have been measured during the lag phase. (Martin)

Cultures, established from the isolates of *Nitzschia pungens* f. *multiseries* from several locations around Nova Scotia, yielded domoic acid under stressful conditions. This suggests the potential for the occurrence of toxic algal episodes in the Scotia-Fundy Region. Observations on its growth characteristics, existence of morphological variants, peculiarities of its life cycle, photosynthesis-photon flux density relationships, and production of domoic acid demonstrate that *Nitzschia pungens* f. *multiseries* is a unique alga. (Subba Rao)

Experiments have been conducted to compare the production of PSP toxins by *Alexandrium excavatum* (from Gaspé) and *Alexandrium fundyense* (from the Bay of Fundy) in axenic and non-axenic cultures. The results suggest that bacteria do not play a direct role in the production of toxins, but that their presence in culture does stimulate the growth rate of the dinoflagellates and the amount of toxin produced. It therefore appears that bacteria play an important, but indirect role in the production of PSP toxins. (Stewart)

- Uptake, Storage, and Depuration of Toxins by Marine Organisms

Protocols have been developed for experimental feeding of bivalve molluscs on cultures of *Nitzschia pungens* sufficient to yield commercially significant levels of contamination by domoic acid. The preliminary results indicated that accumulation may result from physiological feedback processes; i.e., at high input levels domoic acid may suppress depuration (Silvert and Subba Rao 1992). *N. pungens* has no measurable effect on the filtration or feeding rates of *Mytilus edulis*, nor are there any histologically detectable neurological effects in either *M. edulis* or *Argopecten irradians*. Depuration of domoic acid in mussels from levels approaching 100 µg/g to below 20 µg/g can be accomplished in less than 48 hours in recirculated, ultraviolet light-sterilized seawater at 8°C. (Scarratt)

Lobsters (250-300 g) were held in individual compartments with flowing sea water at ambient temperature (4-12°C). Digestive glands of scallop, *Placopecten magellanicus*, containing PSP toxins (400 µg STX equiv./100 g wet weight) were fed to the lobsters two times per week for 16 weeks. PSP toxins were detected in the stomach and hepatopancreas but not in the tail muscle. The mean concentrations of PSP toxins in the hepatopancreas were not significantly different in lobsters sampled from Week 2 through to Week 16 (>500 µg STX equiv./100 g wet weight, range 275-3200 µg STX equiv./100 g wet weight). (Haya)

- Effects of Toxins on Marine Organisms

A technique to measure the effects of toxic microalgae on *in vitro* salmonid hearts has been rejected. Reproducible baseline electrocardiograms could not be obtained. Subsequently, prototype equipment for the *in vivo* measurement of physiological and behavioral effects of harmful algae on fish has been designed and assembled. This bioassay will use a surgically implanted acoustic heart monitor. (Wildish)

Kainic acid and domoic acid were found to be lethal by interperitoneal injection to Atlantic salmon smolts (200-300 g) at dosages of 30 and 3 mg/kg, respectively. All deaths occurred within 8 hours after administration. (Haya)

- Fate of Toxins

An extensive array of bacteria isolated from various coastal marine environments were examined, using a Warburg respirometer, to determine the capacity of bacteria to oxidize domoic acid. Very few bacteria showed any activity. In fact, the respiratory activity of virtually all of the bacteria was markedly inhibited by domoic acid. In growth experiments, domoic acid affected most bacteria negatively. (Stewart)

- Physical and Chemical Oceanography

The 1990 data collected by the long-term temperature monitoring program (LTTM) were published (Gregory et al. 1991), as well as a report reviewing the results of the first 10 years of the program. (Petrie)

Data from the phytoplankton monitoring program and the LTTM program were examined to document water exchange in selected coastal inlets. A method of evaluating exchange based on heat content was developed and tested. The development and testing of a simple shelf upwelling model was begun. (Bugden)

Physical data collected as part of the phytoplankton monitoring program were organized, checked for accuracy, and archived. Thermograph moorings were maintained at all sampling sites. Data quality was maintained by a regular program of field and laboratory calibrations. Seabird CTD firmware and software were upgraded, and the instrument was fitted with a dissolved oxygen sensor. "Pop-Up" float development and testing was continued. Field tests with the Vemco thermographs were initiated. (Budgen)

Under the Classification of Estuaries, Inlets and Coastal Embayments Program (CEICE), the geometry of 120 inlets from the Scotia-Fundy and Gulf Regions has been digitized. A user interface for the database has been developed and tested. (Petrie)

The particle size frequency of suspended particulate matter at phytoplankton monitoring stations has been determined and data are currently being analyzed. This work is being conducted to understand the possible role of flocculation processes in the dissipation of algal blooms. (Kranck)

Anomalous nutrient concentrations have been observed at Ship Harbour in the late summer and early fall as part of the phytoplankton monitoring program. A series of detailed surveys was initiated in 1991 to map the spatial extent of these anomalies in the inlet. (Strain)

Newfoundland Region

- General

The Newfoundland Region has no formal phycotoxins projects at this time. In November 1991, there was a problem with PSP along the Atlantic coast of the northern peninsula. Inspection Services Branch had to close shellfish beds for over 1 month. No known toxin-producing organisms could be found in the water column, but dinoflagellate cysts were observed in sediments.

DFO

NATIONAL WORKING HYPOTHESES FOR PHYCOTOXINS RESEARCH

The PWG developed a list of scientific hypotheses to help guide the evolution of the DFO phycotoxins research program over the next 5 years. While most of these general hypotheses are pertinent to all DFO Regions, different priorities among Regions may shape Regional programs differently.

The proposed working hypotheses address two major aspects of the marine phycotoxin problem as follows:

A. ORIGIN OF TOXIC EVENTS

This group of hypotheses addresses issues related to the dynamics of toxin-producing algae and the biotic and abiotic factors promoting their growth pattern and production of toxin.

The basic hypothesis is that there is an increase in the diversity and frequency of toxic algae problems in Canadian waters which parallels a similar world-wide pattern.

Causes and means are still elusive and the subject of much scientific debate. Several sub-hypotheses should be tested:

Hypothesis A1: Nutrient enrichment from human activity (industrial effluents, agricultural run-off, soil erosion and run-off following removal of vegetation, municipal sewage, aquaculture, etc.) in fresh and coastal waters is increasing primary productivity and algal biomass, modifying species composition, and increasing the frequency, magnitude, and duration of algal blooms in the coastal zone:

- the ratio of inorganic macronutrients (N:P:Si) affects and/or controls the species composition of natural phytoplankton populations and, ultimately, the production of phycotoxins by toxigenic species; and

- the combination of increased inorganic and organic nutrient loadings and modified nutrient ratios plays a major role in promoting abnormal blooms.

Hypothesis A2: Modifications of the physical and chemical environment through natural events can promote harmful blooms if potentially deleterious species are present:

- in the short-term, abrupt localized atmospheric events promote the occurrence of harmful algal blooms by modifying physical (temperature, salinity, stratification, advection, etc.) and chemical (nutrient loading and ratios) environmental characteristics; and
- in the longer term, global climate changes will alter productivity patterns and phytoplankton species composition in Canadian estuarine and coastal waters.

Hypothesis A3: The progression of harmful blooms, the initiation of toxin production, and the dependence of toxicity on the strain of the isolate are controlled by internal physiological and biochemical processes:

- bacteria and/or viruses play an important role in the production and decomposition of certain phycotoxins; and
- the ability to produce a given toxin can be transferred from one species to another through viral and/or bacterial infestation.

Hypothesis A4: Passive introduction of undesirable organisms, including harmful phytoplankton species, results from some human activities:

- ship ballast waters represent an important vector for introducing non-native species of viruses, bacteria, phytoplankton, zooplankton, invertebrates, and fish, some of which could be undesirable if established in local waters (cyst-forming toxigenic phytoplankton species are especially good candidates for successful "relocation"); and
- transfer of indigenous and native species and the introduction of non-indigenous species are potential vectors for passive transfer or introduction of species of microorganisms and algae.

Hypothesis A5: The proliferation of harmful algal blooms can be caused in part by a decline in herbivorous pelagic and benthic grazers, perhaps in a species-specific manner:

- both anthropogenic and natural influences on the marine ecosystem are adversely affecting the natural recruitment of zooplankton and bivalve herbivores; and
- herbivorous zooplankton and benthic bivalves are preferentially grazing on non-toxic algae, thus allowing toxic algae to flourish with increased production of resting cysts in a cumulative seasonal cycle.

B. EFFECTS OF PHYCOTOXINS

This group of hypotheses addresses issues related to the direct (toxic) and indirect (biochemical and physiological) impacts of phycotoxins on both invertebrate and vertebrate marine organisms at all levels of the food web.

Hypothesis B1: Phycotoxins have deleterious effects on both invertebrate and vertebrate marine organisms including larval and juvenile stages and, therefore, have the potential to disrupt natural food webs and affect the recruitment patterns of commercially exploited fish species.

Hypothesis B2: Herbivorous zooplankton represent an important vector in the transfer of phycotoxins in food webs.

Hypothesis B3: Toxin-producing algae directly affect the feeding behaviour of herbivorous zooplankton and filter-feeding bivalves as well as the vertical distribution and migration of invertebrate zooplankton.

Hypothesis B4: The economic viability of some commercial fisheries is limited because of human health concerns related to phycotoxins.

With sufficient knowledge of relevant physical, chemical, and biological factors identified in the above hypotheses, toxic events can be described and explained in multidisciplinary numerical models. If developed far enough, numerical models could also have the potential to predict toxic events.

These two groups of working hypotheses do not cover the entire range of questions, concerns, and activities associated with the DFO phycotoxin program. For example, they do not explicitly refer to the need for the continuing provision of analytical support and for alternate toxicity testing methods to the mouse bioassay. Nevertheless, they do reflect the extensive experience that DFO scientists have accumulated over the past 3 years and provide a sound basis for determining future research priorities.

PUBLICATIONS

The following list contains the publications that have resulted from the DFO phycotoxins research program since 1986 to April 1992.

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Acknowledgements

The program for this workshop was developed by a local Scientific Committee from the Maurice Lamontagne Institute with the following membership:

Serge Demers
Jacques Gagné
Maurice Levasseur
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Responsability for local arrangements and secretarial work was efficiently handled by Manon Laflamme, assisted by Lise Levesque. Several other IML staff members participated in one way or another to the organisation of the workshop. Of mention is the contribution of Lionel Corriveau who expertly managed the audio-visual presentation aspects of the Workshop as well as the social and coffee break sessions. Financial support was provided by the Biological Sciences Branch of the Québec region, Maurice Lamontagne Institute, Department of Fisheries and Oceans.

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